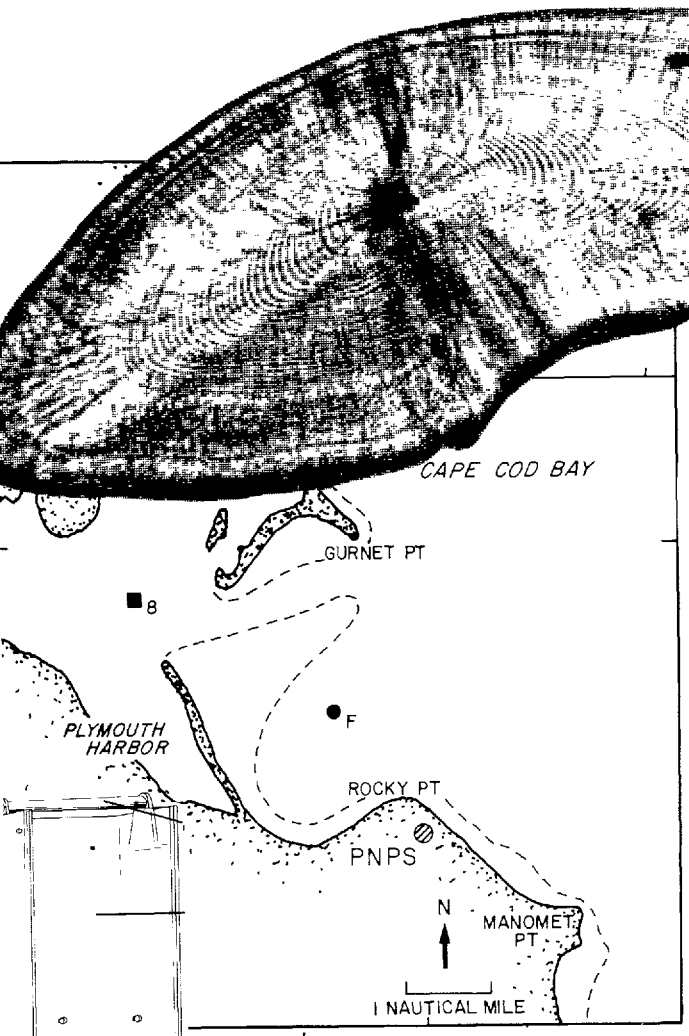
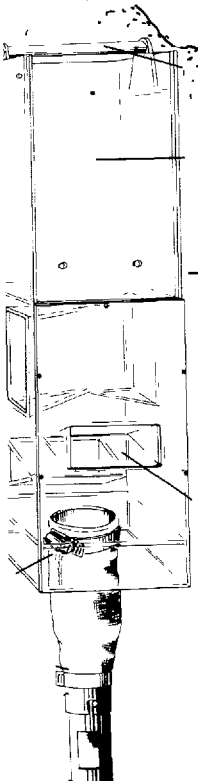
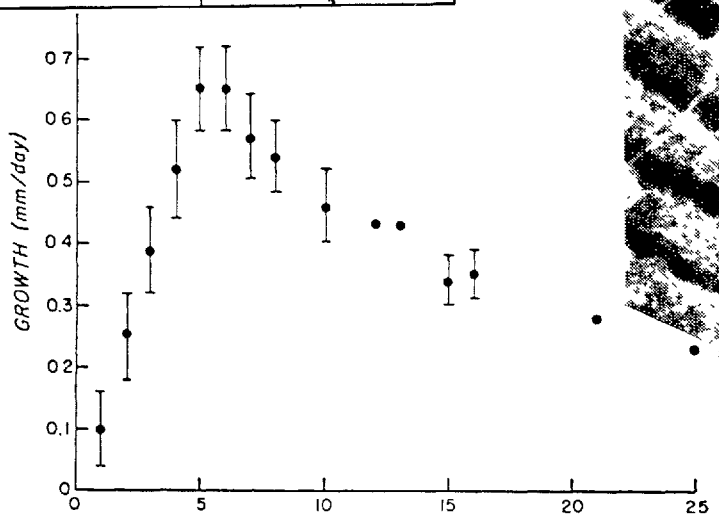
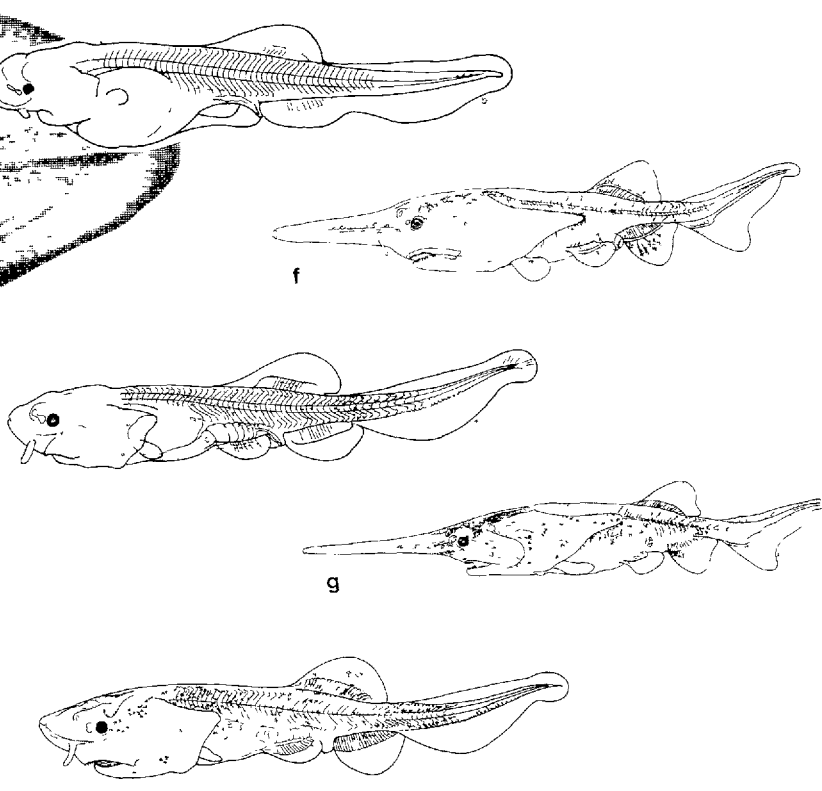
LEE A. FUIMAN

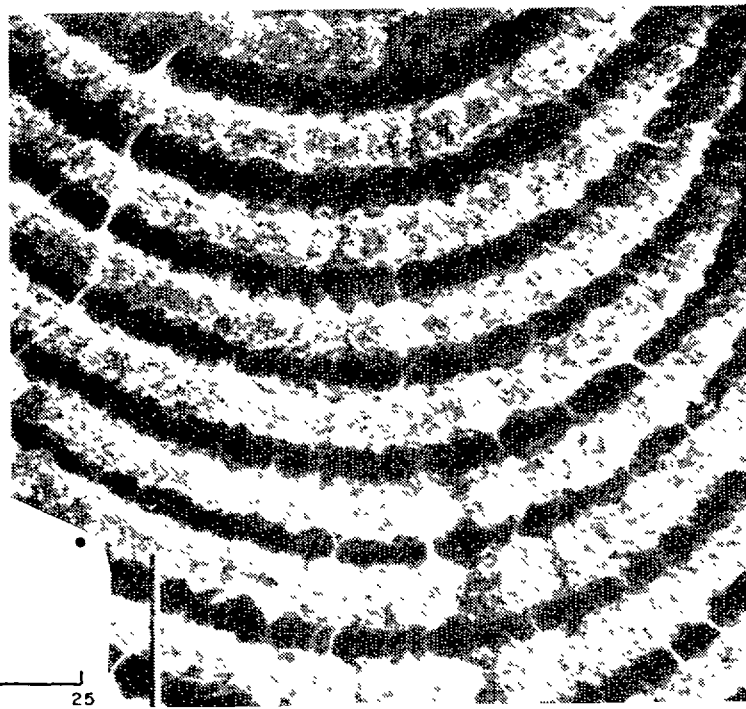
Fifth Annual Larval Fish Conference











THE FIFTH ANNUAL LARVAL FISH CONFERENCE

C. F. Bryan, J. V. Conner, F. M. Truesdale
Editors

Proceedings of a Conference held at the
Louisiana State University, Baton Rouge
Baton Rouge, Louisiana

2-3 March 1981

Louisiana Cooperative Fishery Research Unit
and
The School of Forestry and Wildlife Management

THE FIFTH ANNUAL LARVAL FISH CONFERENCE

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PREFACE

The Fifth Annual Larval Fish Conference was a success for many reasons, foremost among which was the enthusiastic participation of 107 registrants who came from 31 of the United States and five Canadian provinces. Roughly two-thirds of the participants were from academia (happily including many students), while the remainder came in equal proportions from industry (consulting firms and utilities) and state or federal conservation agencies. The meeting featured 24 contributed papers and 15 posters, dealing mainly with growth estimation; distribution; feeding ecology; sampling methodology; and descriptive morphology of freshwater, estuarine, and marine fishes. Some of the contributions were withheld for publication elsewhere or other reasons but many, after peer-review and editing, appear in this volume. We gratefully acknowledge the special efforts of our reviewers, who are listed below, as well as the cooperation of the authors, who must at times have felt that they were being alternately ignored and harrassed.

Special thanks are also due to the coordinators of our three informal workshops: Bruce D. Taubert ("Use of otoliths for daily-age estimation in fish larvae"); Daniel J. Faber and Perce M. Powles ("Larval fish sampling in lotic environments"): and Bruce W. Stender ("Identification of western Atlantic sciaenid larvae").

Assistance in planning the conference was provided by the hosts of previous meetings: Bob Wallus, Bob Hoyt, Ron Fritzsche, and Lee Fuiman. We appreciate the logistical support received from the following departments/organizations and individuals: Louisiana Cooperative Extension Service (Larry de la Bretonne and C. D. McKerley); LSU Division of Continuing Education (Dean W. W. Hymel and Mrs. Mabel C. Downs); LSU Department of Zoology and Physiology (Dr. K. C. Corkum, Gary Fitzhugh, John Scheide, and Sallie White Scheide); and the Louisiana Department of Wildlife and Fisheries (Mark Chatry). Many LSU fisheries students deserve thanks: Tim Bosley, Brian Boyer, Jim Ditty, Gary Hutton, Mike Millard, Maurice Muoneke, Marcos Velez, and Steve Zimpfer. Barbara Bryan and Kitty Conner helped with registration, coffee breaks, and airport transportation.

Entertainment was coordinated by Dan C. Brazo, whose dignified ascent to the podium from the men's room was clearly the highlight of the jambalaya banquet.

It was our pleasure to host the Fifth Annual Larval Fish Conference and to edit these proceedings. We assume full responsibility for errors and omissions.

John V. Conner

DEDICATION

To Carol W. Fleeger and Steven J. Levine, without whose tireless efforts and enthusiasm there never would have been a Fifth Annual Larval Fish Conference ...

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DAILY GROWTH OF WINTER FLOUNDER (*Pseudopleuronectes americanus*) LARVAE IN THE PLYMOUTH HARBOR ESTUARY

RICHARD L. RADTKE AND MICHAEL D. SCHERER

ABSTRACT

The formation of daily otolith increments was documented in larval winter flounder (*Pseudopleuronectes americanus*) which made it possible to assess the age and growth of field-collected larvae. In reared larvae, increment deposition occurred on a daily basis after yolk sac absorption. Daily growth rates in length from field-collected specimens changed in accordance with the age of the larvae. Daily growth (in length) was found to be greatest 5 to 7 days after yolk sac absorption and thereafter decreased with an increase in age. The larvae from field collections conformed to a logarithmic (ln) transformation of the independent (increments) variable with a resultant equation of $Y = 1.66 + 2.16 \ln X$. With these data the potential exists to compare population growth of different larval fish populations.

INTRODUCTION

The ability to discern absolute age of fish larvae captured from the field is a prominent problem in larval fish research. In the past, age calculations and growth estimates were based principally on length frequency distributions. For example, Sameoto (1972) and Boyar et al. (1973) followed length frequency modes through time in order to calculate growth of herring larvae, *Clupea harengus harengus*. However, length frequency techniques require large samples and thus may be prone to sampling error. Often the length frequencies observed are not true reflections of the age and growth of larval fish (Radtke 1978). Certainly, it is important that aging techniques for larval fish be as accurate as possible.

Daily increments have been found in the otoliths of many larval fish, and it has been postulated that these exist in all larval fish otoliths. Pannella (1971, 1974) was the first to describe daily increments in fish otoliths. Subsequent works by Brothers et al. (1976), Ralston (1976), Struhsaker and Uchiyama (1976), Taubert and Coble (1977), Barkman (1978), Radtke (1978, 1980), Radtke and Waiwood (1980), Schmidt and Fabrizio (1980), and Steffensen (1980) found daily increments in otoliths from a myriad of fish species. These studies have contributed to a better understanding of the population of dynamics of each species studied.

The application of daily otolith increments to discern population dynamics must be performed with discretion. Studies by Brothers et al. (1976) and Radtke (1978) demonstrated that daily otolith increments form at different developmental times; i.e, some species may hatch with increments already formed while other fish species may not form increments until yolk sac absorption. Hence, age at formation of the first otolith increment must be documented for each species before precise age determination can be accomplished.

We attempted to document the formation of otolith increments in winter flounder larvae (*Pseudopleuronectes americanus*) in order to define growth patterns in wild larvae.

MATERIALS AND METHODS

Larval winter flounder of known age were obtained from laboratory cultures. Artificially fertilized eggs were obtained from adults collected in Narragansett Bay, Massachusetts. Eggs were passed through a fiberglass window screen to separate adhesive clumps, and held in aerated, static 10-liter containers. Approximately 75% of the water, maintained at ambient temperatures (5-8°C), was exchanged two to three times per week. A 12L/12D light regime was maintained with fluorescent Gro-lux bulbs; absolute darkness was avoided by using a 15w incandescent bulb as a night light. Beginning seven days after hatching the rotifer (*Brachionus plicatilis*) and green alga (*Dunaliella* sp.) were introduced to the rearing containers (Houde 1972). Brine shrimp nauplii (*Artemia* sp.) were also added 10 to 12 days after hatching. Although prey densities were not monitored, they probably exceeded two per ml. Approximately 10 larvae were fixed in 95% ethanol for otolith analysis on a daily basis from hatching through day 20 and again on day 34.

Wild larval flounder were collected on two dates in May 1980 at stations located in the Plymouth Harbor, Duxbury Bay estuary (station 8), the Plymouth Bight area of Cape Cod Bay, and, on the second date, the discharge canal of the Pilgrim Nuclear Power Station (PNPS, Fig. 1). Water temperature and salinity ranged from 8-12°C and 28.0-28.5 °/oo. Collections were made with a 0.333-mm mesh, 3/4 m Tucker net (Tucker 1951 and Clarke 1969)

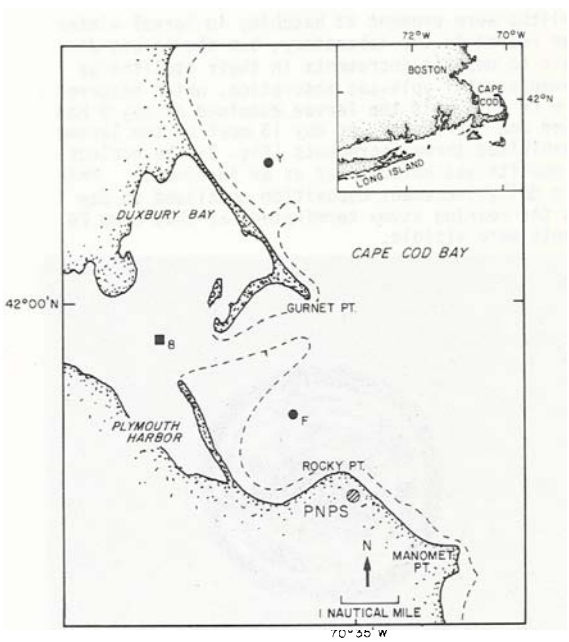

Figure 1. Sampling stations located in Plymouth Harbor, Duxbury Bay, and Cape Cod Bay.

towed off the side of a 12-m dragger at 2-2.5 knots. All tows were oblique and, based on readings from a General Oceanics 2030 flowmeter, sampled approximately 175 m^3 of water in a 6-minute tow. Samples were preserved in 95% ethanol after removing as much seawater as possible. Larval flounder were removed from the samples within 36 hours of collection and transferred to fresh 95% ethanol.

Before otoliths were removed, total lengths were measured (± 0.1 mm), excess ethanol was allowed to

evaporate and larvae were submerged in glycerol to clear the specimens and make the otoliths visible. All three otoliths (sagitta, lapillus, and asteriscus) were extracted where possible. However, only sagittae were used for increment determinations. Otoliths were washed with 95% ethanol, dried, mounted on glass slides with Flo-Texx (Lerner Laboratory, Stamford, Connecticut), and viewed and photographed at 1000X under a light microscope.

Increment counts were taken directly from the photographs. Each photograph was numbered and the combined photographs were shuffled. Thus otolith increments were counted without knowledge of which larva was being analyzed. Three counts were made of each otolith. If two of the counts were identical, that increment count was accepted. If all three counts were within two of each other, the average was accepted. When the counts did not fit into either of these categories, the sample was rejected. The use of such a count regime resulted in a 5% rejection rate.

Sagittae from reared samples were also viewed with a Scanning electron microscope (SEM) in order to examine internal structure and to validate the light microscope counts. Each dried sagitta was attached to a SEM viewing stub with 5-minute epoxy and then ground slightly with one-micron diamond polishing compound (Buehler Ltd. Evanston, Illinois). The otolith was then etched with 7% EDTA (Disodium ethylene diamine tertaacetate) (pH 7.4, adjusted with NaOH) for 1-5 minutes, coated with gold and viewed.

RESULTS

Otoliths were present at hatching in larval winter flounder reared in the laboratory, but the larvae did not begin to deposit increments in their otoliths as daily events until yolk-sac absorption, which occurred on day 9 or 10. Half the larvae examined at day 9 had deposited one increment. At day 13 most of the larvae (80%) exhibited three increments (Fig. 2; the nucleus of the otolith was not counted as an increment). This trend in daily increment deposition continued to day 34 when the rearing study terminated; at that time 24 increments were visible.

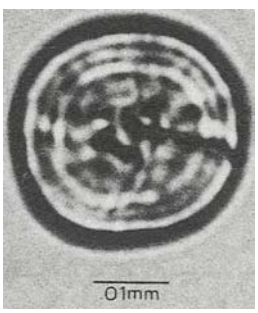

Figure 2. Sagitta from a 13-day-old reared winter flounder larva (Pseudopleuronectes americanus) showing three increments.

Scanning electron microscope investigations on reared larvae validated the light microscope observations. The rugose surface of the etched otolith provided a detailed image of the otolith increments (Figs. 3 and 4). The increments surveyed contained only a small amount of calcium carbonate (as aragonite) in comparison to the large portion of protein matrix (Degens et al. 1969). The protein matrix is clearly visible in Fig. 3, and the small holes present in the decalcified matrix are spaces in which the aragonite crystals had formed. SEM techniques make it conceivable to study microstructural disruptions as they relate to a larva's past growth history.

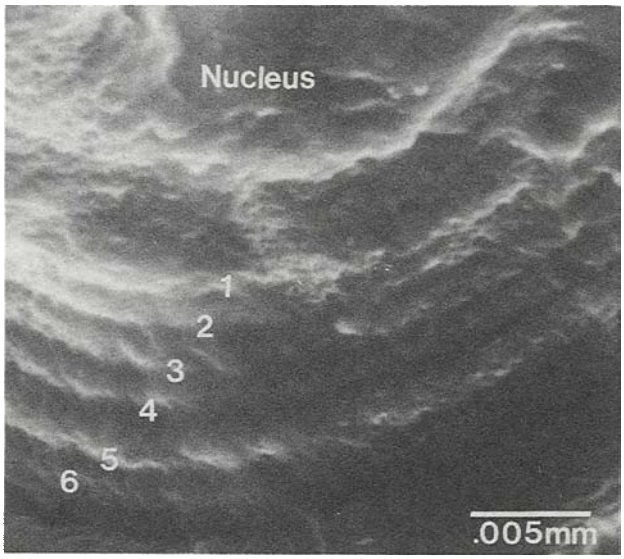

Figure 3. Scanning electron micrograph of a sagitta from a 16-day-old winter flounder larva showing six distinct protein ridges. Otolith is surrounded by epoxy resin.

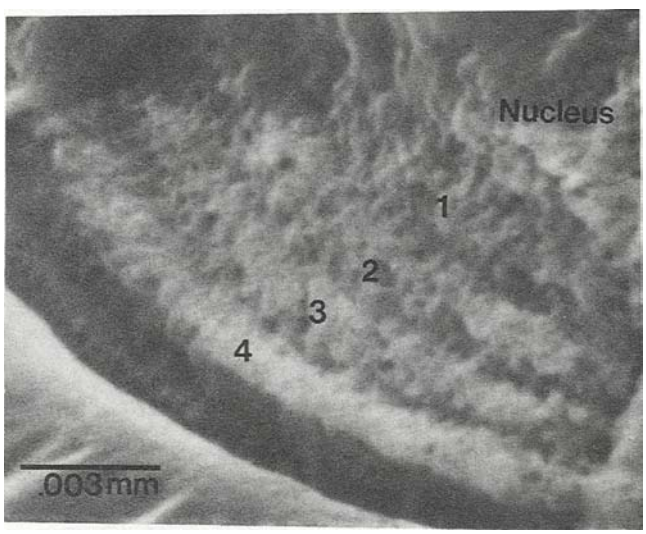

Figure 4. Scanning electron micrograph of a 14-day-old winter flounder larva which displays four protein ridges. Small holes are present in the protein matrix where aragonite crystals of calcium carbonate were deposited.

Based on several tests with laboratory-reared flounder larvae, we found that larvae placed in 95% ethanol while alive did not shrink unless they were fairly large in size. Samples of larvae with a total length of 3.9 mm (8 days old) and 4.7 mm (19 days old) showed no shrinkage in mean length while samples at 7.1 mm (37 days old) decreased in total length by only 4%. Since this shrinkage factor was small, and only 13% of our field-collected larvae were greater than 7.0 mm, we did not make adjustments for this effect.

Increment counts provided an age estimate of the time from yolk-sac absorption to capture of wild larvae.

The increments found in wild larvae proved to be more discrete than those exhibited by reared speciments (Figs. 5 and 6). A comparison of length frequency of the 120 wild larvae with increment frequency displayed different patterns (Fig. 7). The length frequency histogram revealed little except that the fish appeared to fit a normal distribution while the increment frequency histogram indicated that most flatfish in the samples were less than 10 days past yolk-sac absorption.

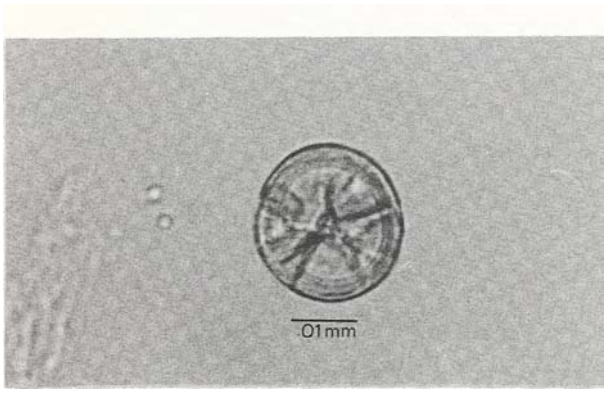

Figure 5. Sagitta from a field-collected larval winter flounder (TL = 3.5 mm) showing four increments.

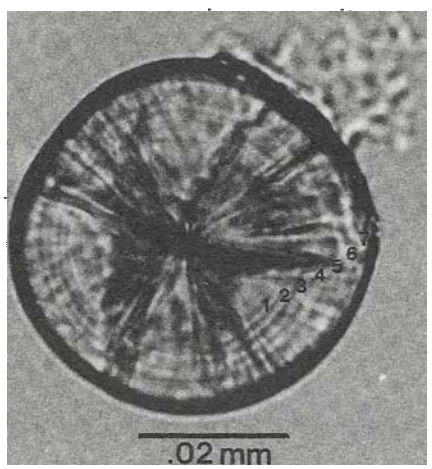

Figure 6. Sagitta from a field-collected larval winter flounder (TL = 5.5 mm) with seven increments present.

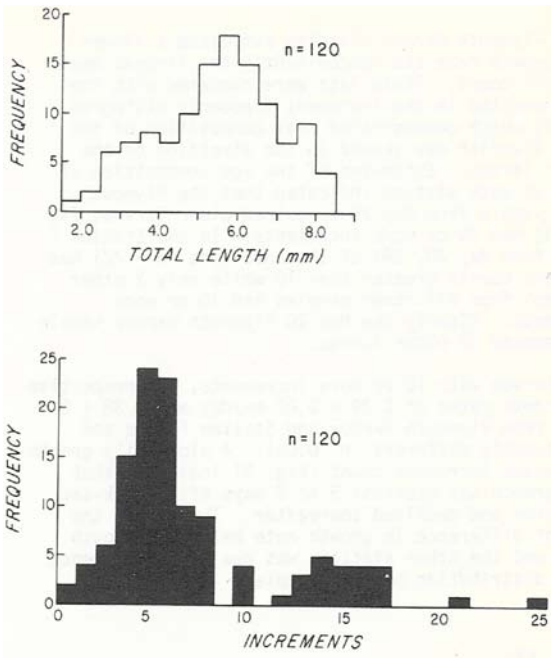

Figure 7. Length and increment frequencies of wild winter flounder larvae.

Daily growth rate was calculated for each larva and then averaged for each station sampled (Table 1). Since wild larvae exhibited increment formation at 2.5 ± 0.3 mm, this length was assumed to be the length at initial increment formation and was subtracted from the total length of all larvae before daily growth rates were calculated. Mean increment numbers were also computed for each station to provide an estimation of the age structure for each sample (Table 1). Mean daily growth rate and mean increment counts were compared between stations within each date using a one-way analysis of variance. (Station Y from May 20 was excluded because of the small sample size, n = 4.) No significant differences were found among the May 5 data, but among the May 20 data significant differences ($p < 0.01$) were detected between both variables. A subsequent SNK (Student Newman Keuls) test indicated that mean daily growth rates differed significantly between the station 8 and both Station F ($p < 0.01$) and the PNPS station ($p < 0.05$). Growth was not significantly different ($p < 0.05$) between Station F and PNPS. Mean increment counts were significantly different among all stations (the difference between the PHDB station and PNPS station was significant at $p < 0.001$; all other differences were at $p < 0.05$).

Table 1. Sample size, average daily growth (mm/day), and average increment number for larval winter flounder collected by station and date, May 1980.

Date	Station	n	Average Daily Growth	Standard Deviation	Average Increments	Standard Deviation
May 5	8	17	0.63	0.19	5.4	1.9
	F	19	0.53	0.29	5.3	1.4
	Y	16	0.62	0.11	5.9	1.5
May 20	8	23	0.38	0.08	12.4	4.5
	F	22	0.57	0.21	8.6	4.9
	Y	4	0.63	0.04	6.5	0.6
	PNPS	19	0.50	0.22	5.2	5.0

The Plymouth Harbor flatfish exhibited a slower daily growth rate and concomitantly the largest mean increment count. These data were compared with the data presented in the increment frequency histogram (Fig. 7) which demonstrated that composition of the larval flatfish was skewed in the direction of the younger larvae. Estimates of the age composition of larvae at each station indicated that the Plymouth Harbor sample from May 20 contained older larvae; 74% (n = 23) had 10 or more increments. In the Station F sample from May 20, 36% of the specimens (n = 22) had increment counts greater than 10 while only 2 other specimens from all other samples had 10 or more increments. Clearly the May 20 Plymouth Harbor sample was composed of older larvae.

In larvae with 10 or more increments, the respective mean growth rates of 0.39 ± 0.07 mm/day and 0.38 ± 0.1 mm/day from Plymouth Harbor and Station F were not significantly different ($p > 0.05$). A plot daily growth rate versus increment count (Fig. 8) indicated that daily growth was greatest 5 to 7 days after yolk-sac absorption and declined thereafter. Therefore, the apparent difference in growth rate between Plymouth Harbor and the other stations was due to a difference in age distribution between samples.

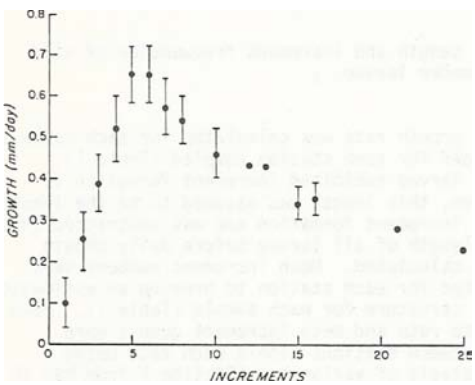

Figure 8. Differences in cumulative daily growth compared with age (increments) after yolk-sac absorption in wild larval winter flounder. Mean ± one standard deviation. Points without bars represent single specimens.

Consequently, all samples were pooled and the length and increment data fitted to a logarithmic (ln) growth function of the form $Y = 1.66 + 2.16 \ln X$ ($r = 0.87$) where X equals the increment count and Y the total length (Fig. 9).

DISCUSSION

Otoliths are the first calcified tissues to form in developing fish embryos and are a prominent and easily observed structure in numerous developmental studies (Armstrong and Child 1965, Long and Ballard 1976, and Radtke 1978). Recent studies of otoliths from larval fish have shown that, while otoliths may always be present at hatching, species differ in the number of increments present at that time. Brothers et al. (1976) and Radtke (1978) found that two to four increments may form prior to hatching in species which have relatively large eggs and long incubation periods such as the grunion (Leuresthes tenuis) and mummichog (Fundulus heteroclitus). Those species with small eggs and short incubation periods examined by Brothers et al. (1976) such as the northern anchovy (Engraulis mordax) did not begin to deposit increments until yolk-sac absorption was complete. The winter flounder have comparatively small eggs (0.7-0.9 mm) and a relatively long incubation period (15 days at 6-8°C), and apparently began to deposit daily increments after yolk-sac absorption.

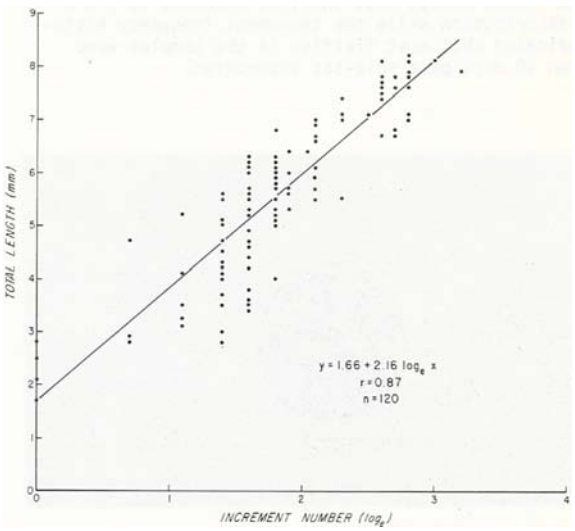

Figure 9. Regression of total length (mm) versus increment number ($\log_e$) for wild winter flounder larvae.

The otoliths of winter flounder larvae were not heavily calcified, and banding in the protein matrix was clearly evident in SEM preparations (Figs 3 and 4). The protein matrix in fish otoliths is postulated to act as a template for crystalization of calcium carbonate in aragonite form (Degens et al. 1969) and our data from decalcified otoliths of larval winter flounder support this. The spaces between the protein ridges were locations of calcium carbonate precipitation. It seems that the increments apparent in larval fish otoliths are caused by changes in protein formation. Through the measurement of protein ridges in SEM preparations, exact distances could be measured for back calculations of growth. Struhsaker and Uchiyama (1976) used otolith increment distances in larval nehu (Stolephorus purpureus) to accomplish back calculation, although their light microscope methods could be erroneous due to the diffraction of light. SEM preparations would not be subject to the bias of light diffraction and therefore have the potential to yield more precise data.

Mean growth rate after yolk-sac absorption in samples of larval winter flounder from different areas of Cape Cod Bay ranged from 0.38 to 0.63 mm/day. These growth rates were similar to those of northern anchovy at 8 mm which ranged from 0.34 to 0.55 mm per day (Methot and Kramer 1979), but were greater than those for redfish larvae (Sebastes spp.) which ranged from 0.12 to 0.18 mm/day (Radtke 1980).

Methot and Kramer (1979) found that shrinkage did not occur among northern anchovy larvae unless they died near the beginning of a 6-minute plankton tow. Our tows were all relatively short, 4-5 minutes, which would have kept a similar type of shrinkage factor to a minimum. Nevertheless, the mean size at yolk-sac absorption found among wild larvae in this study (2.5 mm) appears to be small relative to the size of our reared larvae at yolk-sac absorption (3.9 mm). This may reflect true variations among larvae developing under different conditions or suggests that shrinkage among net-captured larvae should be examined in detail, particularly among larval flounder collected in power plant discharge canals where the larvae are more likely to be dead before capture and preservation.

Growth in length in larval winter flounder increased from yolk-sac absorption to 5 or 6 days beyond that point and then declined steadily at least until 25 days after yolk-sac absorption (Fig. 8). Our growth estimates were 0.10 mm/day at day 1, 0.65 mm/day at day 5 and y, and 0.23 mm/day at day 25 beyond yolk-sac absorption. Laurence (1975) found no general trend in specific growth in weight among winter flounder larvae from yolk-sac absorption to metamorphosis at 2, 5, and 8°C. He did, however, present data from Mulkana (1966) which suggested that specific growth may decrease with increasing age. The change in body shape which is apparent in developing larval winter flounder appears consistent with those findings. Growth in total length slows at about the time growth in body depth increases as larval flounder gradually change from a pelagic larva to a benthic flatfish. Laurence's (1975) data on specific growth in weight suggests that growth in depth compensates for the decline in growth in length so that little change occurs in growth in weight.

Our data indicate that increment counts in larval flounder otoliths are useful in determining daily growth rates and could be useful for back calculating daily growth rates. It would be interesting to compare the growth rate of larval winter flounder in Plymouth Harbor, Duxbury Bay, and Cape Cod Bay with larval flounder populations from other areas.

ACKNOWLEDGEMENTS

Thanks are due to Dr. Vern Barber and the staff of the electron microscope laboratory, Biology Department, Memorial University, Newfoundland, for their help on SEM techniques and for providing SEM time, and to N. Clark for typing. This research was supported by a visiting fellowship from the National Research Council of Canada to R. Radtke and by a contract with Marine Research, Inc., supported by Boston Edison Company.

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GROWTH EFFICIENCY ESTIMATES FOR LABORATORY-REARED LARVAL SPOTTED SEATROUT FED MICROZOOPLANKTON OR ROTIFERS

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ABSTRACT

Spotted seatrout eggs were obtained from adult females injected with human chorionic gonadotropin. Larvae were fed wild microzooplankton or laboratory-cultured rotifers (Brachionus plicatilis) until 12 days after hatching (metamorphosis). The microzooplankton diet was tested at 24, 28 and 32°C, and the rotifer diet was tested only at 28°C. Larval spotted seatrout feeding rates were estimated at prey concentrations of 25, 100 and 1,000 per liter. Average gross growth efficiency estimates (K_1) for zooplankton-fed larvae ranged from 19 to 80%, those of rotifer-fed larvae ranged from 17 to 80%, those of rotifer-fed larvae ranged from 17 to 96%. Larvae fed zooplankton exhibited a trend for the highest K_1 with increasing prey levels at 28°C and 32°, whereas larvae fed rotifers trended toward highest K_1 at 25 prey per liter.

INTRODUCTION

Fishery scientists recognize recruitment failures, but lack a clear understanding of the mechanisms that determine year-class strength in pelagic fish populations (Lasker 1978). Recent investigations of marine fish larval survival, and factors affecting it, may offer possible approaches to solve the problem (Hunter 1976, Houde and Taniguchi 1979). It is generally accepted that the most significant mortality of marine fish populations occurs during the pelagic larval phase. Thus, the population dynamics of marine fishes are inseparably related to density-dependent and density-independent regulatory mechisms acting on embryos and larvae (Gulland 1965, Cushing 1974, 1976, May 1974, Hunter 1976). It has been suggested that recruitment could be regulated during the early life of larvae (Cushing and Harris 1973, Harris 1973, Jones 1973, Lasker 1978). Models have been developed (Jones 1973, Cushing and Harris 1973, Beyer 1980, Beyer and Laurence 1980) in which density-dependent mortality was suffered if larvae failed to capture a specific number of prey, or if larvae did not grow rapidly enough (a function of prey availability) to reduce their probability of capture by predators.

Growth efficiency may be used as one index of how fish larvae are coping with their environment. Conover (1978) concisely defines K_1 as the fraction of rations which appears as growth. My study examined some variables that have an effect on gross growth efficiency in a subtropical marine fish, the spotted seatrout, Cynoscion nebulosus. The effects of prey type, prey concentration and temperature on growth efficiency were examined.

METHODS

Adult spotted seatrout were captured during their spawning season in South Biscayne Bay, Florida and transported to the laboratory within 4 hours after capture. I adapted well-known techniques using HCG injections (Stevens 1966, 1970; Haydock 1971; Colura 1974; Hirose et al. 1979) to induce oocyte maturation and hydration in spotted seatrout. A 1-ml disposable tuberculin syringe with a 25-gauge, 15.9-mm long hypodermic needle was used to administer lyophilized HCG dissolved in Holtfreter's saline. To successfully induce oocyte maturation and ovulation, females were injected if biopsied ovarian fragments contained vitellogenic oocytes within the follicles that were 0.40 mm diameter, but preferably when they were greater than 0.45 mm diameter. A single injection of 1 to 1.5 IU HCG per gram body weight was injected intramuscularly into the hypaxial musculature beside the first dorsal fin.

Eggs were fertilized by the dry method in a clean dry dish. Seawater of the exact experimental test temperature and salinity was slowly added to the dish. Dead gametes, cellular debris and, in instances where eggs were extruded by hand, blood clots, mucous and feces were removed. Care was taken to avoid changes in salinity and temperature of the water. Eggs were kept in the fertilizing dishes and placed in constant temperature water baths for the initial incubation. Eggs could be transferred to other containers or tanks without inducing mortality or deformities, if they were transferred before germinal ring development or after gastrulation. Embryos surviving gastrulation and showing normal development were selected for experiments.

Wild microzooplankton, predominantly copepod nauplii and small copepods <150 µm breadth were tested as prey types at 24, 28 and 32°C. Laboratory-cultured marine rotifers, Brachionus plicatilis, were used as prey at the optimum 28°C temperature (Taniguchi, unpublished data) for rearing larval spotted seatrout.

Zooplankton were collected in 53 µm mesh 0.5-m diameter plankton nets suspended from a pier. Multiple daily collections of fresh zooplankton were used for all rearing and feeding rate experiments. Net mesh sizes of 280 µm, 110 µm and 53 µm were used to grade zooplankton before feeding to larvae. More than 95% of the organisms retained on the 53 µm mesh were copepod nauplii or copepodites of approximately 35-130 µm breadth, and was the first size fraction fed to spotted seatrout larvae. Occasionally, protozoans, predominantly tintinnids, were abundant. Mean dry weight of an individual of the 53 µm fraction was 0.15 µg (about 8×10^{-4} cal). The intermediate-size zooplankters retained by the 110 µm mesh netting were mostly large copepod nauplii, copepodites, microcopepods, and an occasional barnacle nauplius. Mean dry weight of an individual in this fraction was 0.51 µg (about 29×10^{-4} cal). This fraction was added to the spotted seatrout larva diet, together with the smallest fraction 7 days after hatching. Mean dry weight of plankton sampled from rearing tanks being fed the 53 µm and 110 µm mesh fractions was 0.29 µg per prey (Houde 1978). Those retained on a 280 µm mesh netting were mostly large copepods, barnacle cyprus larvae, decapod zoea and chaetognaths. This fraction was used only in some preliminary experiments when laboratory-cultured spotted seatrout were raised beyond the larva to juvenile transformation stage.

At the optimum 28°C temperature (Taniguchi, unpublished data), the laboratory-cultured rotifer, Brachionus plicatilis, was tested as a larval food. Concentrations of rotifers were selected on the basis of their reported dry weights (Theilacker and McMaster 1971). Rotifer mean dry weights (0.16 µg) were equivalent to the mean dry weights of copepod nauplii (0.15 µg) in the sieved fraction retained by the 53 µm mesh. Thus, in experiments where larvae were fed rotifers, their concentrations were maintained at the same nominal concentration during the second to seventh day after hatching as in comparable experiments using copepod nauplii. At 7 days after hatching, when mean dry weight per prey increased to 0.29 µg in the zooplankton diet experiments (Houde 1978), the mean concentration of rotifers was doubled. In this way the mean dry weight of rotifers per liter that was potentially available to larvae was equivalent to the dry weight available in experiments using zooplankton.

Rearing tanks were all-glass rectangular aquaria of

76-liter capacity. Seawater was drawn from Biscayne Bay, and filtered through dacron filter wool and 35 µm Nitex netting before being added to the tanks. Dense phytoplankton inocula of Chlorella sp. and Anacystis sp., with cell densities of $9x10^5$ to $30x10^6$per ml were added to each tank at the beginning of an experiment at a dose of 3.3 ml of each phytoplankter concentrate per 1,000 ml of culture water. Phytoplankton numbers usually increased from $9x10^4$ to $50x10^4$ cells per ml in the rearing tanks during the first 4 days. Thereafter, algal cells were maintained at high levels ($1x10^5$ to $3x10^5$ cells per ml) by daily additions of the phytoplankton cultures (up to 2.5 ml per 1,000 ml of rearing water) if the numbers diminished. Seawater was not filtered or recirculated during the experiments. However, 10 to 20% of the tank volume was exchanged on alternate days.

Larvae were reared at 24, 28 and 32°C. Two hundred embryos were stocked per 76 liters. This method produced enough larvae for the feeding rate experiments, even when they were reared under potentially stressful conditions of low temperature (24°) and low prey concentrations (25 per liter).

Larvae in the rearing tanks fed ad libitum during the light photoperiod. Prey concentrations were maintained at nominal prey levels of 25, 100 and 1,000 organisms per liter using the method of Houde (1977, 1978). Prey levels in tanks were determined 3 to 6 times daily from organism counts in aliquots. Aliquots of 50 ml for 1,000 nominal prey per liter, 100 ml for 100 nominal prey per liter, and 200 ml for 25 nominal prey per liter were obtained by quickly aspirating seawater from three random places in the tank. Duplicate aliquots allowed a ± 20% precision for mean prey concentration estimates. Prey concentrations were adjusted 3-6 times during the day to maintain nominal concentrations, depending on counts of prey in the tanks.

Prey were first offered when eye pigmentation and concurrent mouth development permitted larvae to begin feeding, usually early in the morning of the second day after hatching at 28 or 32°C and before noon of the second day after hatching at 24°C. Preliminary experiments showed that spotted seatrout completely transformed from larva to juvenile by 12 days after hatching at the optimum temperature (28°) and 100 per liter prey concentration. After transformation virtually all spotted seatrout survived. Therefore, 12 days was selected as the experimental period.

Temperature was controlled by 100 or 150-watt immersion heaters in the tanks and seldom varied by more than ± 0.2°C from the designated experimental temperature. Temperatures were checked and adjusted 2 to 3 times daily.

Salinity was maintained at 33 ± 2 °/₀₀ in rearing tanks, the usual salinity in Biscayne Bay during most of the spotted seatrout spawning season. Salinities were checked on alternate days with an optical refractometer and deionized or tap water added as needed.

Flourescent lights illuminated the rearing tanks. Larvae were exposed to a 13:11 hour light: semi-dark cycle simulating the natural diurnal period during the middle of the spotted seatrout spawning season. Two 61 cm, 20-watt "Cool-white" flourescent tubes in a single light fixture were suspended 15 cm above the tank water surface. These flourescent tubes emitted 11,000 lux, and 4,000 lux reached the water surface. For comparison, the brightest incident sunlight in Miami, Florida, during the winter was 110,000 lux. During the summer it was 116,000 lux. Illumination reaching the bottom of the 76-liter tanks, filled with filtered seawater containing phytoplankton, was 2,500 lux.

Lights over the rearing tanks were extinguished at night, but laboratory room lights were left on. Night-time illumination in the tanks ranged from 200 to 700 lux for the brightest impinging light on any tank rack; however, the average incident light reading for any tank was about 100 lux.

Daily rations of spotted seatrout larvae were estimated from replicated experiments in 10-liter rectangular glass tanks into which larvae were transferred. These tanks were illuminated and set up exactly as were rearing tanks. The 10-liter tanks were filled with filtered seawater and adjusted to the desired test temperature before prey were added. Prey concentrations were estimated by aspirating 200 ml aliquots. When four consecutive aliquots showed that prey number was at the desired density, phytoplankton was added and 3 to 30 larvae were transferred individually to the tank. The total water volume in the tank was measured to estimate the total number of prey available for larvae to consume. After 2 to 13 hours of feeding, before larvae were removed from the tank, four 200-ml aliquots were taken to estimate the number of prey remaining. Larvae were permitted to feed for up to 13 hours (their rearing photoperiod) so long as prey numbers were not reduced to less than the natural logarithm value of the starting prey concentration. Experimental feeding periods were shortest for large larvae, which consumed prey at a rapid rate. The difference between prey numbers at the beginning and at the end was the total prey consumed by the larvae. After each ration experiment the larvae were sacificed, measured, dried for 24 hours at 70°C and weighed.

Gross growth (or energy conversion) efficiency (K_1), values were calculated from Ivlev's first order coefficient of food utilization:

$$K_1 = \frac{\Delta W}{R \Delta t}$$

where K_1 = Gross growth efficiency, ΔW = Total dry weight gained during the experimental period t days, and R = Dry weight daily ration.

Estimates of larval spotted seatrout rations showed the 24-hour dry weight ration (R) was changing daily as larvae grew. I used daily estimates of ration obtained from the fitted ration model

$$R_t = R_0 e^{ct}$$

where R_t = Dry weight ration consumed in 24 hours at age t, R_0 = Theoretical estimated initial dry weight ration at age zero, c = exponential coefficient, and t = Age in days after hatching, to estimate the cumulative ration consumed by larvae during an 11-day period for 2 to 12 days old larvae. The average K_1 for the 11-day period was estimated from a derivation of Ivlev's relationship:

$$K_1 = \frac{W_{t_{12}} - W_{t_2}}{\sum_{t_2}^{t_{12}} R_t}$$

Where K_1 = Average estimated K_1 for an 11-day period, W_{t_2} = Dry weight of a larva at 2 days after hatching (the first day a larva was capable of capturing prey), $W_{t_{12}}$ = Dry weight of a larva at 12 days after hatching, the approximate time metamorphosis was complete, and R_t = Estimated 24-hour ration at age t (days after hatching).

RESULTS AND DISCUSSION

The equations describing the relationship between the ration consumed in 24 hours and the age of the spotted seatrout larva are summarized in Table 1. These relationships estimated the cumulative 11-day ration (Table 2) for 2 to 12 days old spotted seatrout. The dry weight growth between 2 and 12 days old larvae is summarized in Table 3.

Average gross growth efficiency estimates (K_1) of microzooplankton-fed larvae ranged from 19 to 80%, those of rotifer-fed larvae ranged from 17 to 95% (Table 4). The K_1 estimates were averaged for the 11-day larval phase from 2 through 12 days after hatching. Some estimates of K_1 appeared high (those > 60%). The 95% efficiency of rotifer-fed larvae at the 25 per liter prey level probably is far too high, but the reason for this result is unclear. Larvae fed microzooplankton exhibited a trend for the highest K_1 estimates at the highest prey levels, within a temperature group, whereas larvae fed rotifers showed the opposite trend of high K_1 at the lowest prey level (Table 4). The range of the estimated K_1 values increased for microzooplankton-fed larvae as temperature increased. The uniformly high K_1 estimates at 24°C suggests that larvae utilized (assimilated) captured prey quite well at low temperatures. The relatively low efficiency at 32°C, and

Table 1. Summary of equations describing the ration consumed per larva per 24 hours (R_t) for 2 to 12 days old (t) spotted seatrout larvae. The ration, R_t, was coded by adding the value 1.

Temperature (°C)	Prey Concentration (Numbers per liter)	Ration Equation	Standard Error of Regression Coefficient	Number of Observations (n)	Coefficient of Determination (r^2)	Total Number of Larvae tested
		MICROZOOPLANKTON PREY				
24	25	$R_t+1 = 0.88e^{0.25t}$	0.048	13	0.71	185
24	100	$R_t+1 = 1.12e^{0.35t}$	0.062	14	0.72	177
24	1000	$R_t+1 = 3.14e^{0.39t}$	0.088	12	0.66	133
28	25	$R_t+1 = 0.61e^{0.53t}$	0.053	12	0.91	141
28	100	$R_t+1 = 2.78e^{0.43t}$	0.070	12	0.80	130
28	1000	$R_t+1 = 42.73e^{0.28t}$	0.026	12	0.92	134
32	25	$R_t+1 = 4.24e^{0.30t}$	0.082	7	0.73	74
32	100	$R_t+1 = 12.37e^{0.35t}$	0.045	6	0.94	57
32	1000	$R_t+1 = 58.61e^{0.31t}$	0.031	6	0.96	55
		ROTIFER PREY				
28	25	$R_t+1 = 6.18e^{0.16t}$	0.040	10	0.68	119
28	100	$R_t+1 = 22.42e^{0.18t}$	0.076	8	0.48	96
28	1000	$R_t+1 = 118.27e^{0.13t}$	0.027	7	0.82	83

Table 2. The cumulative ingested dry weight of food for spotted seatrout larvae from 2 to 12 days after hatching (cumulative 11-day ration). The areas beneath the exponential functions describing ration consumed per larva per 24 hours (Table 1) were integrated to obtain the cumulative 11-day ration.

Temperature (°C)	Prey Concentration (Numbers per liter)	Total 11-day Ingestion per larva (µg)
	Microzooplankton Prey	
24	25	61.51
24	100	225.80
24	1000	1388.64
28	25	811.73
28	100	1431.64
28	1000	4949.11
32	25	564.26
32	100	2845.24
32	1000	8914.37
	Rotifer Prey	
28	25	234.84
28	100	993.38
28	1000	3524.86

Table 3. Estimated growth of spotted seatrout larvae from 2 to 12 days after hatching.

Temperature (°C)	Prey Concentration (Numbers per liter)	Estimated dry weight at 2 days after hatching (µg)	Estimated dry weight at 12 days after hatching (µg)	Estimated weight gain for 11 days (µg)
		MICROZOOPLANKTON PREY		
24	25	9.83	49.44	39.61
24	100	7.76	153.51	145.75
24	1000	8.81	956.20	947.39
28	25	10.44	425.06	414.61
28	100	12.25	449.65	437.41
28	1000	18.56	3603.82	3585.26
32	25	10.33	115.04	104.71
32	100	14.51	1008.52	994.01
32	1000	24.32	7142.44	7118.12
		ROTIFER PREY		
28	25	12.26	237.43	225.18
28	100	14.14	305.77	291.63
28	1000	18.66	600.47	581.81

Table 4. Summary of gross growth efficiency estimates (K_1) for larval spotted seatrout reared in the laboratory. The estimates were calculated from the predicted cumulative 11-day ration (Table 2) and predicted larval dry weights (Table 3).

Temperature (°C)	Prey Concentration (Numbers per liter)	Estimated K_1
	Microzooplankton Prey	
24	25	0.64
24	100	0.65
24	1000	0.68
28	25	0.51
28	100	0.31
28	1000	0.72
32	25	0.19
32	100	0.35
32	1000	0.80
	Rotifer Prey	
28	25	0.96
28	100	0.29
28	1000	0.17

low prey density (25 per liter), suggests poor food utilization under those conditions. But, as prey levels increased at 32° the gross growth efficiency of larvae increased markedly.

Some of the K_1 estimates summarized in Table 4 were considerably higher than expected. Estimated values summarized by Conover (1978) and Brett and Groves (1979) for invertebrate and vertebrate marine zooplankton were lower. The expected K_1 values were observed to range from 20 to 50% (Conover 1978). One possible cause of my high K_1 estimates was that I may have underestimated the true consumed ration. The more likely cause of low ration estimates was the selection for larger-than-average prey by the spotted seatrout larvae. The mean cumulative ration ingested by a larva from 2 through 12 days after hatching was estimated based on the mean weights of zooplankton and rotifers in the 53 µm and 110 µm-mesh size classes. Houde (unpublished data) estimated one standard deviation to be 0.10 µg for the 0.15 µg mean weight of 53 µm-size zooplankton prey fractions, and one standard deviation to be 0.14 µg for the 0.51 µg mean weight of 110 µg-size zooplankton. If spotted seatrout larvae consumed prey, on average, one standard deviation larger in weight than the mean prey weight, then the K_1 estimates would be decreased considerably. I recalculated a few ration models based on prey weights of 0.25 µg and 0.65 µg for the 53 µm and 100 µm-size prey fractions, respectively. These new ration estimates were used to recalculate the cumulative ingested ration, which was used to recalculate K_1. Estimates of K_1 were decreased about 30 to 40% from the estimates based only on mean prey weights. This suggests that additional studies of prey weights, prey selectivity and prey size preference as obtained from stomach analyses of larval spotted seatrout will be very valuable for metabolism and bioenergetic studies. My K_1 estimates are the best available estimates of spotted seatrout larvae. Refinements in technique and experimental design, e.g., testing an individual larva and determining prey-size selectivity with age, will produce more accurate K_1 estimates.

Gross growth efficiency may be the simplest and most meaningful indicator of an adequate diet, ration level, state of health, and suitability of an artificial environment for a fish (Brett and Groves 1979). Highest gross growth efficiencies (65 to 80%) occur during yolk utilization by embryos; however, high values in caloric equivalents have been reported for post-embryonic stages, ranging from 50 to 60% (Hatanaka and Takahashi 1956, Brett and Groves 1979). Growth efficiencies estimated for spotted seatrout larvae tended to be high at the 3 temperatures and 3 prey concentrations tested for a zooplankton diet, and it was high at 28° and 25 prey per liter for the rotifer diet. The estimated values may not be absolutely accurate, but trends are apparent.

The test prey levels of 25, 100, 1,000 per liter ranged from the low to the high concentrations of microzooplankton in coastal waters reported by Houde and Taniguchi (1979). Prey density provides a measure of prey available for capture by larvae, but it does not directly provide a measure of energy available for growth. Spotted seatrout larvae raised at 24° evidently cannot benefit from increasing prey concentrations. The smallest larvae were observed in the 24° experiments (Table 3). It is possible that their metabolism was so reduced that larvae tested at the 25 per liter prey concentration were ingesting and assimilating nearly as many prey as the larvae tested at 1,000 per liter prey concentration. It appears that gross growth efficiency was strongly influenced by the digestion and assimilation rates at the low temperature. The quantity of prey captured was low and, due to the inverse relationship between number of prey captured and the degree of digestion of ingested prey, the growth efficiencies were uniformly high. Thus, the average estimated K_1 values may have stayed within the range of 64 to 68% due to the decreased metabolism of larvae in these experiments. Spotted seatrout larvae consuming microzooplankton at 28° appeared adept at utilizing their ingested ration. A K_1 of 51% reflects the good feeding ability of larvae surviving the low 25 per liter prey concentration. During the 11-day period larvae with marginal feeding abilities probably died in the rearing tank, and the surviving larvae used in the 25 prey per liter feeding rate experiments were probably the exceptional ones able to effectively feed at low prey levels. The results of the 32° experiments supported this view; there was no clear difference between K_1 values at 28° and 32° for prey concentrations of 100 and 1,000 per liter (Table 4). The low K_1 of 19% for the 32°, 25 liter prey level indicated high metabolism at the elevated temperature. Metabolic demands might increase significantly at high temperatures and lead to less food energy to be available for growth. Only at prey levels approaching 100 per liter might the 32° growth efficiency increase above those efficiencies observed at 28° (Table 4). At the 100 per liter prey level spotted seatrout at 32° may encounter sufficient food for maintenance and growth and reflect this in the growth efficiency estimates.

The rotifer diet produced a decreasing trend in K_1 values as prey concentration was increased at 28°C. The very high K_1 of 96% observed at the 25 per liter prey concentration is perplexing. But, the decreasing K_1 trend can be explained by the inability of spotted seatrout larvae to consume larger quantities of rotifers to equal the ration that could be consumed by larvae offered zooplankton at the same temperature and prey level.

Juveniles and adults may survive long periods of time when provided with a maintenance ration, but the maintenance ration concept appears not to exist for larvae. Larvae must grow to survive. As a larva develops, behaviorally and physically, its capabilities for capturing, handling and ingesting larger prey increase. If larger prey are not available, more energy is expended per food item in searching and capture, as reflected in the rotifer experiments. I examined some effects of prey concentration and ration on K_1 of spotted seatrout larvae. In the subtropics critical prey concentrations for a K_1 value may change and not be constant for spotted seatrout larvae, the prey requirement would change with the temperature.

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A TECHNIQUE FOR THE EXAMINATION OF OTOLITHS BY SEM WITH APPLICATION TO LARVAL FISHES

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ABSTRACT

A technique is presented which simplifies the preparation of small otoliths for examination in the scanning electron microscope (SEM). Otoliths or entire larvae may be infiltrated with a low viscosity embedding medium, sectioned, sanded and polished easily. The polished surface is then etched to bring out the increment pattern which can be observed by SEM. The materials needed for this technique are inexpensive and readily available.

INTRODUCTION

In recent years several studies have been published that demonstrated the presence of daily growth increments in otoliths (Pannella 1971 1974; Brothers et al. 1976; Struhsaker and Uchiyama 1976; Taubert and Coble 1977; Barkman 1978; Schmidt and Fabrizio 1980; Wild and Foreman 1980; Radtke and Dean in press). Both light microscopy and scanning electron microscopy have been used to observe these increments.

The light microscope has the advantage of requiring little otolith preparation, that is, simply cover dried otoliths with immersion oil (Brothers et al. 1976). However, this method has limitations when either large otoliths or increments near the edge of the otolith need to be observed. Large otoliths can be viewed with the light microscope by grinding and polishing them until a thin section is obtained. However, due to the structural array of the otolith crystals, optical effects can lead to inaccurate counts or make observation of increments near the edge difficult. Use of a scanning electron microscope (SEM) is often necessary to avoid these difficulties. The main advantage of the SEM is that extremely small increments can be resolved easily when otoliths are properly prepared. This paper presents methods for mounting, sectioning, sanding, polishing and decalcifying otoliths. These techniques consistently provide good SEM preparations in the least amount of time.

METHODS

Bluegills (Lepomis macrochirus about 20 mm SL) were used to show differences among decalcifying procedures because the increments of juveniles are distinct. The fish were seined from the edge of a small pond and preserved immediately in 95 percent ethanol. The otoliths were removed later, dried and stored in #3008 Multiwell™[1] tissue culture trays (Falcon Division, Becton, Dickinson and Co., Oxnard, CA).

Otoliths were embedded in the hard formula low viscosity embedding medium reported by Spurr (1969). Spurr embedding kits are available from several electron microscopy supply houses.

Flat embedding molds (#110 Pelco 20 cavity, Ted Pella Inc., Tustin, CA., see Fig. 1) were used to mount otoliths. Prior to placing the otoliths in the mold, the cavities were partially filled with liquid Spurr. The resin was polymerized at 70C for 4 hours or until it was highly viscous. This allowed the otoliths to be properly oriented in the cavities and prevented them from sinking to the bottom of the mold. Each otolith was placed in the partially polymerized Spurr so that a line from the center of the core through the point of the rostrum was parallel to the long axis of the mold cavity (Fig. 2). The mold cavities were then filled with resin and polymerized for 24 hours at 70C.

The hardened blocks containing the otoliths were cut with an Isomet saw[1] (Buehler LTD., Evanston, Ill.) to obtain flat sections. The sections were flattened "boxes" measuring 5 mm x 5 mm x 1-3 mm. The last dimension depended on the size of the embedded otolith.

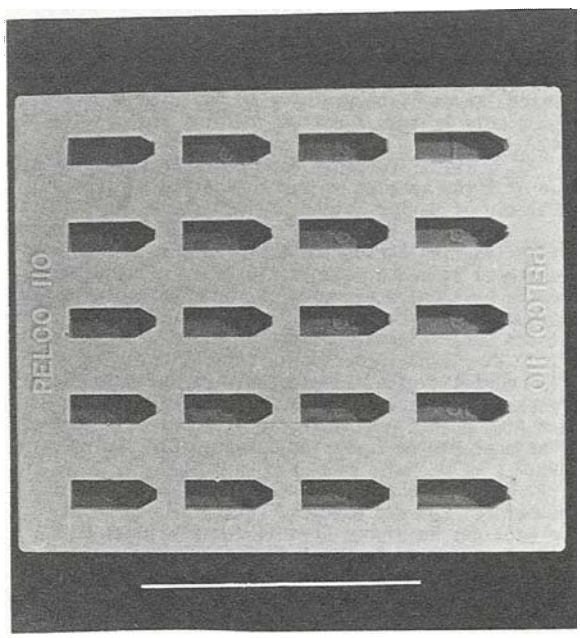

Figure 1. Mold used for embedding otoliths and larvae. Each cavity is 5.0 mm wide and deep, 14.0 mm long. Vertical bar represents 5.0 cm.

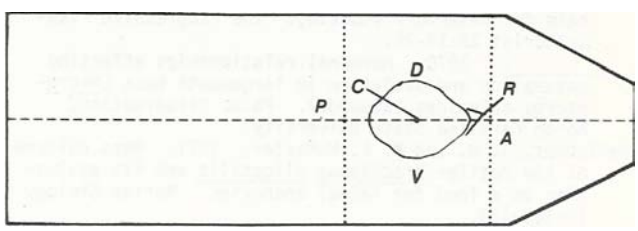

Figure 2. Line drawing showing the orientation of an embedded otolith. Dashed line is the long axis of the otolith, dotted lines indicate where cuts were made to obtain flat sections. A = anterior; P = posterior; D = dorsal; V = ventral; R = rostrum; C = core. The size of the otolith is exaggerated for clarification.

The sections were made by cutting close to the otolith on each side (Fig. 2). The Isomet saw is a convenient tool because it makes a very narrow cut with precise control; however, it is not a necessity for otolith work. We have made satisfactory cuts with a hand coping saw (12.6 teeth per cm). The cuts were made parallel to the transverse plane of the otolith so that the final polished surface yielded a transverse section (see Pannella 1974, 1980; and Taubert and Coble 1977, for diagrams of the otolith planes).

Because the sections were flat, they were easy to

[1]Use of trade name does not imply endorsement by the University of South Carolina.

sand by rubbing against wet 600 grit wet-or-dry sandpaper with a circular motion. For each section, when the core was near the surface of the sanded side, that side was polished against a piece of Microcloth^{R} (Buehler LTD., Evanston, Ill.) containing 0.3 micron alumina polishing compound (Fisher Scientific Co., Fair Lawn, NJ). The section was then rinsed with distilled water and placed on a microscope slide with the polished surface up. Viewed with a light microscope the primordium appears as a dark spot in most L. macrochirus otoliths (Fig. 3). If the primordium was at the surface, the section was cleaned with distilled water in an ultrasonic cleaner. If the primordium was still below the surface, the section was sanded and polished again as needed.

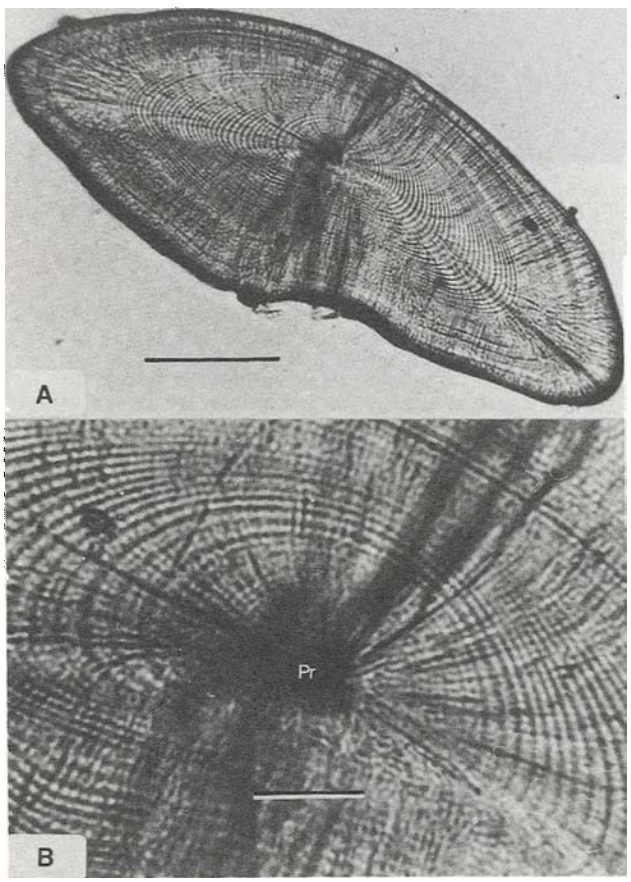

Figure 3. A. Photomicrograph of a sanded and polished L. macrochirus otolith. Horizontal bar represents 100 microns. B. Enlargement of core in A showing primordium (Pr). Horizontal bar represents 20 microns.

After cleaning, the samples were decalcified in one of the following solutions: pH 3.0, HCl for 1, 2, 3, 4, 5 or 10 minutes; 5% disodium ethylenediaminetetraacetate (EDTA); adjusted to pH 7.5 with KOH, for 1, 2, 3, 4 or 5 minutes; 2% aqueous glutaraldehyde (GA) and sucrose (500 mOsm) buffered with 0.1 m sodium cacodylate to pH 7.8 for 1, 2, 3, 4 or 5 hours. After decalcification each sample was rinsed in distilled water and dried. One section was not decalcified to determine the quality of polish.

Warmouths (Lepomis gulosus) were used to show how this technique can be applied to larval fish work. During the summer of 1980 mature warmouths were collected from a pond and stripped in the laboratory. Fertilized eggs were incubated at 25C in a 10 liter aquarium with a photoperiod of 16 hours light and 8 hours dark per day. Larvae were preserved in 95% ethanol 9 days after hatching (approximately 7 mm SL). The larvae were dehydrated for 1 hour in three changes of 100% ethanol. They were infiltrated with resin for 30 minutes in three changes of a mixture of 50% Spurr and 50% ethanol. The remaining alcohol was removed by soaking the larvae in Spurr for 3 hours with 3 changes. The infiltrated larvae were placed in the molds with their long axes parallel to the long axes of the mold cavities. The samples were polymerized, sectioned, sanded and polished (as above). EDTA (5%, pH 7.5) was used for 3 minutes to decalcify the larval otoliths.

All samples were attached to metal stubs and gold coated (100 Å). The specimens were examined in a JEOL JSM 35 or a JEOL JSM U3 scanning electron microscope operated at either 15 or 25 kV.

RESULTS AND DISCUSSION

Hardened Spurr is light yellow and transparent, so that the embedded otolith can be examined at low magnification and progress of sanding can be observed. Figure 4 shows an otolith embedded in the plastic resin and some increments can be seen near the posterior edge. Figure 5 shows an otolith that has been partially sanded, demonstrating that the core can be seen while sanding. Both photographs were made with a dissecting microscope looking through the top surface of the block. The primordium of the otolith must be at the surface after polishing, so frequent inspections are necessary to prevent oversanding.

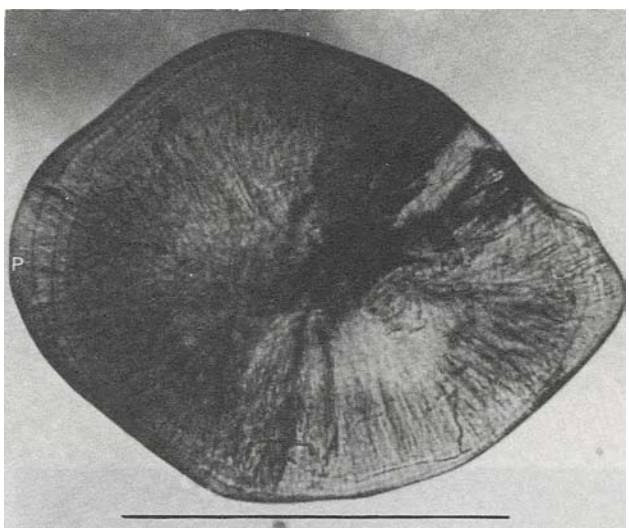

Figure 4. Photomicrograph of an embedded L. macrochirus otolith. Note the increments near the posterior edge. P = posterior. Horizontal bar represents 0.5 mm.

Larvae preserved in ethanol are opaque and their internal features cannot be distinguished. The infiltration process clears the larvae, making their otoliths easy to observe (Fig. 6). Since otoliths of some larvae are very small (e.g. L. gulosus sagittae are approximately 10 microns in diameter 1 day after hatching), their dissection and manipulation is difficult. Also, by orienting the larvae with the otoliths in situ one can consistently section the otoliths in the same plane. Figure 7 shows the otolith of a 9-day old L. gulosus prepared in this manner. Notice that the increments were not distinct. Along with Radtke and Dean (in press) we observed that the increments of fish raised in the laboratory are not as distinct as specimens collected in the field.

The undecalcified polished surface of an embedded otolith gives little information (Fig. 8). With proper decalcification, however (Figs. 9-12), the increment pattern can be seen. Previous investigators (Pannella 1974, 1980; and Brothers et al. 1976) have used 1% aqueous

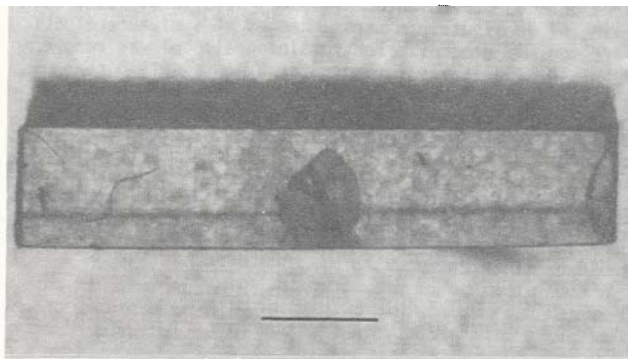

Figure 5. L. macrochirus otolith embedded in a flat section. This otolith has been partially sanded. The photograph was taken through the edge of the section. Horizontal bar represents 1.0 mm.

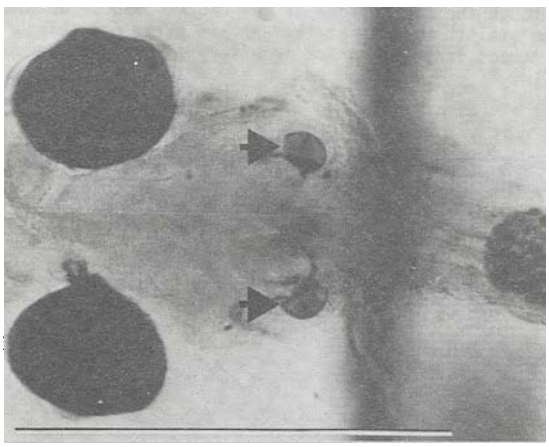

Figure 6. L. gulosus larva (9 day post hatch) embedded in Spurr. The sagittae are marked by arrowheads. Horizontal bar represents 1.0 mm.

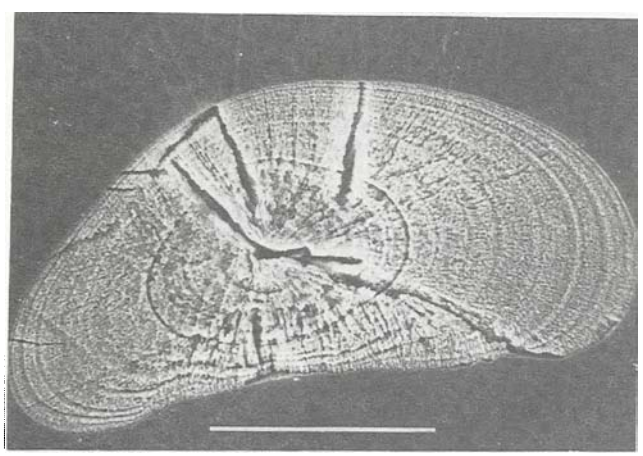

Figure 7. Scanning electron micrograph of a 9 day old L. gulosus otolith. This transverse section was etched for 3 minutes with EDTA. The increments are indistinct as in many lab reared fishes. Horizontal bar represents 50 microns.

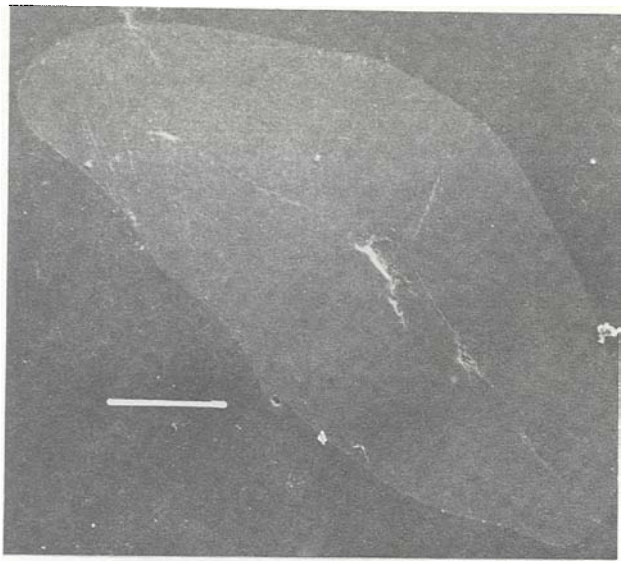

Figure 8. Scanning electron micrograph of an undecalcified L. macrochirus otolith. The outline of the otolith is barely visible and no increments can be seen. Horizontal bar represents 100 microns.

HCl to etch specimens for the SEM. Preliminary investigation in our laboratory indicated that 1% HCl is far too strong and always resulted in a less distinct etch than weaker solutions. Figure 9 shows an otolith etched in pH 3.0 HCl for 4 minutes. The increments are fairly clear. Figure 10 is from the same region of a different otolith etched with HCl for 10 minutes. The increments are less distinct than those of the otolith etched for 4 minutes. An etch time of 4 minutes was the optimum for increment resolution when using HCl (pH 3). Longer or shorter periods of etching produced areas of the otolith which did not show increments.

Both GA and EDTA resulted in more distinct increments than HCl. We compared 4 min. EDTA (Fig. 11) and 3 hour GA (Fig. 12) decalcification to the HCl treatments (Fig. 9 and 10). Notice that in each case the increments were more distinct than the HCl etches. The major difference between EDTA and GA was the time factor. GA was effective with 3 to 5 hours decalcification while EDTA was useful in the range of 1 to 5 minutes.

The best decalcifying solution (EDTA, GA, or HCl) and optimal time varies with species, and even among developmental stages within a species. Therefore, we recommend that each decalcifying solution be tested over a range of time to determine the optimal decalcification time for each species.

By embedding otoliths in Spurr epoxy resin they can be properly oriented and sectioned. This makes sanding and polishing easier and more reliable. Combined with the proper decalcifying technique, this provides more accurate interpretation of otoliths examined in the scanning electron microscope.

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The authors would like to thank Gene Sausser for allowing us to collect fish in his pond. Thanks also to Dave Chestnut and Ray Peeples for help with fieldwork and to Keith Taniguchi and Bobby Ireland for critically reviewing the manuscript. Special thanks to N. Watabe and D. Dunkelberger for technical help on the SEM and to the University of South Carolina E. M. Center and the University of South Carolina School of Medicine for use of their SEM's. This work was supported by the National Park Service Contract No. CX5280-1-0056, the South Carolina Sea Grant Consortium and NSF Grant No. INT781-7742 to Drs. N. Watabe and J. M. Dean.

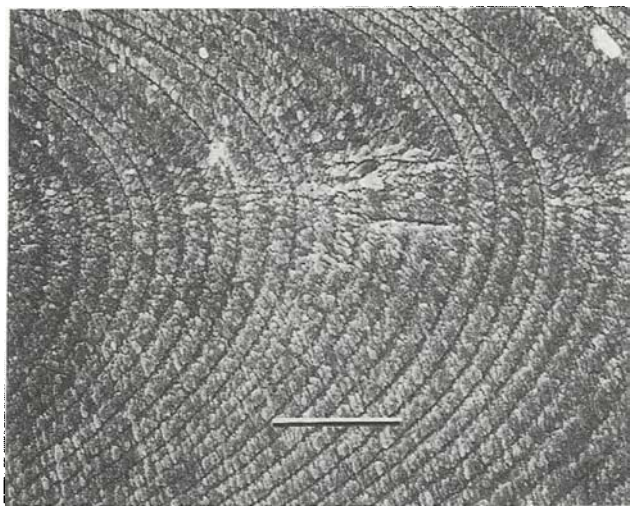

Figure 9. Scanning electron micrograph of a L. macrochirus otolith. Etched in pH 3.0 HCl for 4 minutes. Horizontal bar represents 10 microns.

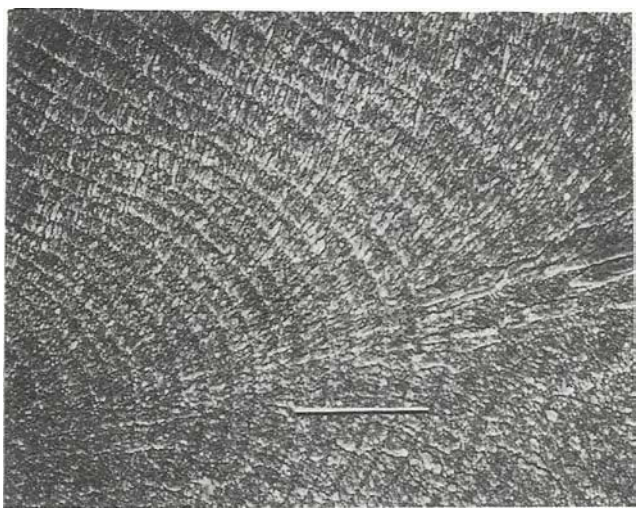

Figure 10. Scanning electron micrograph of a L. macrochirus otolith etched in pH 3.0 HCl for 10 minutes. The increments can be seen but are more distinct in Fig. 9. Horizontal bar represents 10 microns.

Figure 11. Scanning electron micrograph of a L. macrochirus otolith decalcified in EDTA for 4 minutes. The increments are distinct, more than in either of the acid etched specimens. Horizontal bar represents 10 microns.

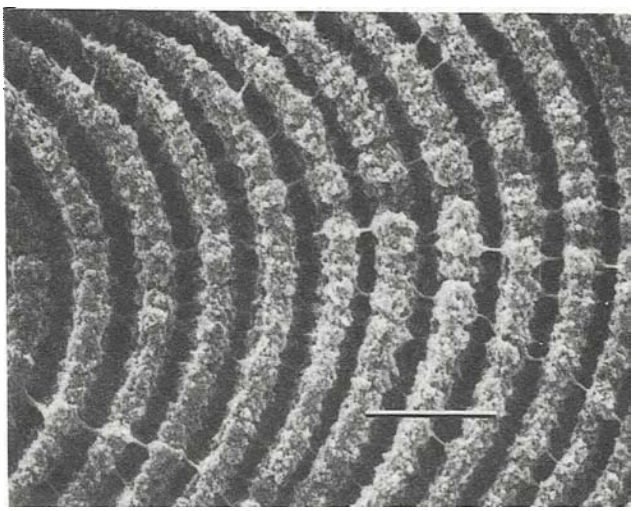

Figure 12. Scanning electron micrograph of a L. macrochirus otolith decalcified in GA for 3 hours. The increments are very distinct, more than Fig. 9-11. Horizontal bar represents 10 microns.

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DECLINE AND CESSATION IN FALL FEEDING OF 0 and 1-YEAR-OLD *Lepomis gibbosus* IN CENTRAL ONTARIO

Nicholas Reid and P.M. Powles

ABSTRACT

Feeding of young *Lepomis gibbosus* (3-7 cm. TL, aged 0 and 1) from Dam Creek (Peterborough, central Ontario) declined perceptibly from late September to early December, 1978. Feeding periodicity was determined by measuring the volume of stomach contents over time. Irregular and temporary rises in feeding were associated with ascents, plateaus, or even slight decreases in the environmental temperature pattern. Young pumpkinseed stopped feeding at a lower water temperature (2°C.) than has been reported for adults of this species in Ontario.

INTRODUCTION

Lepomis gibbosus (Linnaeus), is an omnivorous feeder, taking a variety of food at all depths in the water column (Keast and Webb, 1966). It is one of the most abundant fishes in warmwater ponds, lakes, and streams in east-central North America, and is an important prey of most predatory fishes (Scott and Crossman, 1973). The only detailed food study of pumpkinseed sunfish food habits describes feeding in spring during rising temperatures (Keast, 1978). There are virtually no data on feeding during falling temperatures. Therefore, our information fills the gap on when feeding of "warmwater" species stops in fall or early winter.

To our knowledge, this study also represents the first quantitative documentation of declining fall feeding in a stream population of very young *Lepomis gibbosus*. To monitor the very small changes in amounts of prey consumed, we measured the volumes in capillary tubes. It is hoped that this method will be refined and applied by other workers.

MATERIALS AND METHODS

Samples of *Lepomis gibbosus* were seined between 1300 and 1500 hrs at two locations in Dam Creek, one mile south of Trent University's campus, twice per week from September 21 to December 14, 1978. Five pumpkinseed (under 7 cm. TL) were randomly selected from each sample, suffocated, and preserved in 5% formalin in separate vials after slitting the abdomen. Four Surber samples were taken on the same day from separate locations across the stream, but slightly upriver from the seining area, and were also fixed immediately in 5% formalin.

The fall diel feeding pattern was determined from 120 fish seined at 0100, 0700, 1100, 1500 and 1900 hrs on October 14 and 19, and preserved as previously described. Total lengths (TL) in cm. were recorded, and a scale sample removed to check that only fish of ages 0 and 1 (mean length-range of 3.1 to 6.3 cm.) were included in the study. None of the seined fish appeared to regurgitate their food, although this is a potential problem in food studies (Desselle et al., 1978). Of the 120 fish seined for diel periodicity studies, 60, (or 6 per time interval) were subsampled for food.

Only food from the stomach, and not the intestine, was examined. Stomach content volumes were measured as follows: 1) for 0+ or fish under 4 cm. TL we used blood capillary tubes 100 mm. long (I.D. of 1.6 mm., Kimble Products, Kimax - 51, U.S.A.), in which one end was expanded (Fig. 1) to facilitate introduction of organisms. A plunger (stainless steel pin, with head slightly ground down) weighted with a 1 gm. piece of modelling clay, was inserted and dropped on the food column to remove bubbles. 2) For larger fish, we used a similar method but substituted common laboratory glass tubing of 3.6 mm. I.D. The height of the food column was measured and converted to mm.[3] Only single volume readings were made because successive values were consistently 4-10% lower than the original. This resulted from compaction and deformation of the organisms during handling, and reduced the precision of the volume estimates.[1]

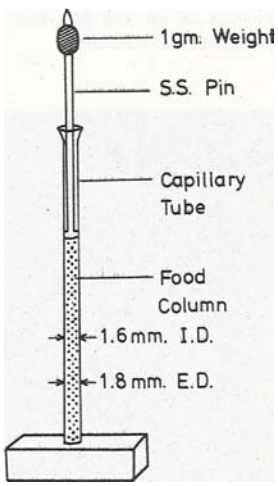

Fig. 1. Capillary tube and stainless steel plunger (pin) used to measure volumes of food in stomachs of postlarval and young *Lepomis gibbosus*. (I.D. = internal diameter; E.D. = external diameter)

Water temperatures were measured with a recording thermometer (W. Lambrecht # 25F, two-probe) at 1500 hrs on each sampling date.

The contribution of each prey species to total stomach volume was estimated by the Swynnerton and Worthington (1940) and Hynes (1970) points method; i.e., one large organism may be equivalent to several smaller ones. The most common taxa were assigned an arbitrary value of one, and the contribution of larger or smaller food items to the total was estimated as a fraction or multiple of one (1.0).

Four Surber samples from each date of fish sampling were combined in one jar to obtain an index of prey availability. Samples were then analysed and the organism

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[1]) Lasenby used horizontal capillary tubes, and measured the change in the meniscuses of water after introduction of organisms.

identified to the lowest taxon possible. Forage ratios were computed as follows:

$$FR_i = \frac{r_i}{p_i}$$

where r_i = proportion of prey i in the diet, and p_i = proportion of prey i in the environment (Surber samples).

RESULTS

Feeding, as measured by stomach content volumes, gradually declined as temperatures fell from 10 to 2°C. during the time period 23 September to 17 December (Fig. 2). Some apparent increases in feeding occurred which were not associated with increasing temperature. Of 7 apparent increases in feeding, only 3 were directly associated with temperature increases (October 14, 22, and November 5). The 4 other pulses (September 27, October 5 and 29, and November 27) were correlated with a slowing down of the overall rate of temperature decline. Feeding ceased at 2°C. in early December at the commencement of ice-up. The lowest temperature at which small Lepomis fed was also 2°C. (December 13). On two occasions after feeding had apparently ceased (November 1 and 15) a slight temperature increase (November 5) and a reduced rate of temperature decline (November 27) induced resumption of feeding. During the first 2 weeks in December young Lepomis ceased feeding at temperatures at or below 2°C.

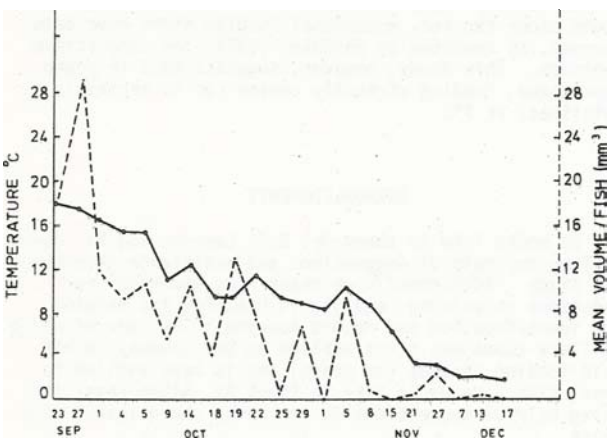

Fig. 2. Average total food volumes of young (0 and 1-yr-old) Lepomis gibbosus in relation to ambient temperatures (N = 113) in Dam Creek, Peterborough, Ontario, 1978.

One feeding peak, probably climaxing around dusk (1900 hrs) appears to be descriptive of the fall feeding activity of young pumpkinseed in the study stream (Fig. 3). After sunset (1800 hrs) feeding apparently diminished and the volume of stomach contents per fish decreased until some time before dawn (0700 hrs), when 86% of the fish had empty stomachs. Thus the choice of a 1300 to 1500 hrs sampling period for fall, fell on a time of average or medium feeding intensity.

Of the four major prey organisms found in the stomachs only Chironomus pupae were relatively uncommon from 1300 to 1500 hrs (Fig. 4). Chironomus larvae, and to a lesser extent, Asellus, were very abundant by 1300 hrs in the stomachs.

After October 25, the food of L. gibbosus was composed mainly of Asellus, Chironomus pupae, Simulium larvae and "other" (or non-identifiable) species, with a proportional reduction in Chironomus larvae from October 9 to early December (Fig. 5).

On 3 occasions, the forage ratios of Tanypus,

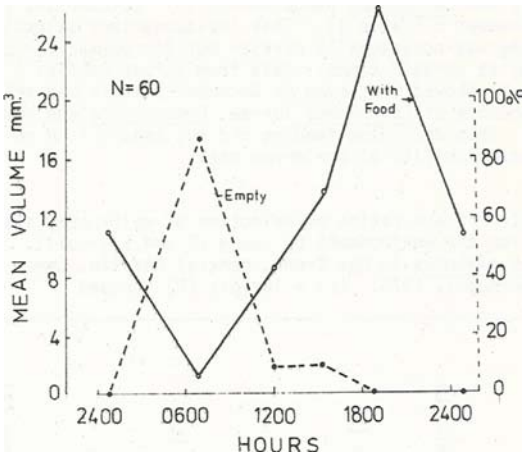

Fig. 3. Changes in feeding activity of young (0 and 1-yr-old) Lepomis gibbosus over two 24-hr periods, October 1978, Dam Creek, Peterborough, Ontario.

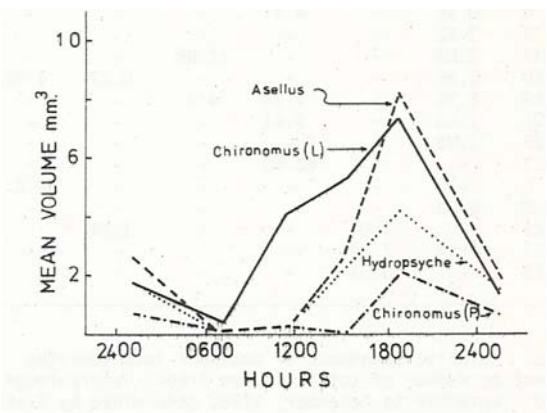

Fig. 4. Prey volumes over two 24-hr periods in the stomachs of 0 and 1-yr-old Lepomis gibbosus from Dam Creek, Peterborough, Ontario. (N = 60).

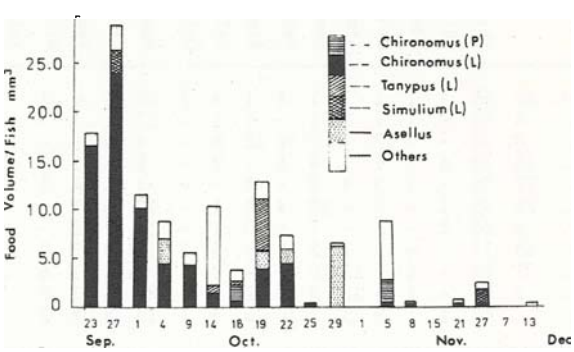

Fig. 5. Seasonal changes in volumes of major prey items found in stomachs of 0 and 1-yr-old Lepomis gibbosus, Dam Creek, Peterborough, Ontario, 1978.

Asellus and Chironomus pupae were high: October 19, 29, and November 5 (Table 1). This indicates that selective foraging was occasionally carried out for particular organisms at certain times. Data from Surber samples (Table 2) showed that even by December 7 there was still an abundance of Chironomus larvae, Hydropsyche and nematodes. Thus declining feeding did not result from reduced availability of preferred prey.

Table 1. Forage ratios of selection of major prey species from the environment by young (0 and 1-yr-old) Lepomis gibbosus in Dam Creek, central Ontario, (near Peterborough), 1978. (L) = larvae; (P) = pupae.

	Chironomus (L)	Simulium (L)	Asellus	Tanypus (L)	Gammarus	Chironomus (P)
Sept. 23	1.34	-	-	-	-	-
Sept. 27	1.34	1.04	-	-	-	-
Oct. 1	1.30	-	-	-	-	-
Oct. 4	0.96	-	4.41	-	-	-
Oct. 9	2.62	-	-	-	-	-
Oct. 14	0.89	-	-	17.86	-	-
Oct. 18	0.86	-	-	-	0.47	1.40
Oct. 19	0.70	-	1.31	4.3	-	-
Oct. 22	1.03	-	0.42	-	-	-
Oct. 25	1.03	-	-	-	-	-
Oct. 29	-	-	62.50	-	-	-
Nov. 5	-	-	-	-	-	50.0
Nov. 8	0.33	-	-	-	-	-
Nov. 21	-	4.04	-	-	1.94	-
Nov. 27	0.14	2.69	-	-	-	-
Dec. 13	-	3.33	-	-	-	-

Table 2. Relative abundance of dominant invertebrates (per cent by number of total) in Dam Creek, Peterborough, Ontario, September to December, 1978, determined by Surber sampler. (L) = larvae; (P) = pupae.

	Chironomus (L)	Chironomus (P)	Odonata	Ephemeroptera	Simulium (L)	Gammarus	Hydropsyche	Asellus	Pelecypoda	Ostrocoda	Nematolde	Elimidae	Tanypus (L)	Hirudinea	Total No. Organisms
Sept. 25	55	1	1	1	3	1	1	1	4	2	22	1	6	1	58
Oct. 4	60	-	1	1	3	1	-	1	1	-	27	1	3	-	39
Oct. 8	33	-	-	12	4	2	10	12	8	-	4	10	-	-	34
Oct. 14	73	6	1	1	1	4	1	-	2	-	5	4	1	1	52
Oct. 22	76	-	1	3	4	3	-	3	-	-	3	4	4	1	39
Oct. 29	45	1	-	7	-	19	4	1	1	-	12	6	1	-	36
Nov. 5	65	2	-	-	-	7	5	3	1	-	15	3	2	-	50
Nov. 15	17	-	-	-	7	12	10	2	9	3	13	3	3	9	33
Nov. 21	21	-	2	-	18	10	29	-	2	1	8	1	1	6	35
Dec. 7	23	-	1	-	12	8	25	2	1	-	21	-	3	2	36
Avg.	47	1	1	3	5	7	10	3	3	1	13	3	2	2	42

DISCUSSION

If stomach volumes can be used to assess extent of feeding, then our study shows that young Lepomis gibbosus ceased feeding at fall temperatures of 2°C. ambient. A decline in feeding was first noticeable in mid-September, immediately after a drop from 18 to 16°C. This decrease in feeding closely followed the declining temperature regime throughout the fall and early winter, with perhaps a few irregularities or inconsistencies. This decline was not associated with reduced availability of prey or seasonal or diel foraging changes by the fish themselves.

Feeding activity of young L. gibbosus in the fall usually peaked once during daylight hours. This maximum volume of food occurred at dusk. Keast and Welsh (1968) report that in summer, adult pumpkinseeds display two peaks in feeding activity, with a maximum at 1800 hrs. This is quite close to the single peak in feeding at 1900 hrs during the fall that we witnessed. Perhaps a slower stomach evacuation in fall (at lower temperatures) may be the reason for the single peak obtained in our study.

In general, the quantity of food consumed over the fall period declined in association with decreasing temperature without drastic changes in types of prey eaten. Hathaway (1927) reported that feeding of L. gibbosus drops off quickly with decreased temperature. Keast (1968) found that large, > 15 cm. L. gibbosus did not feed below 6.5°C., and that at this temperature the stomachs were blocked with mucous. Decline in metabolic rate and in size of the daily ration as fish increase in size has been noted before (Seaburg and Moyle, 1964). Therefore, young pumpkinseed from Dam Creek may have fed at lower temperature (2°C.) to sustain a comparatively higher metabolic rate through the early part of the winter. Although the larger fish in Keast's (1968) study were able to over-winter without late fall-feeding, the smaller, young-of-the-year fish could not.

It is still possible that if this study had been continued under the ice, occasional feeding might have been observed, as reported by Whitaker (1977) for some stream cyprinids. This study, however, suggests that in young pumpkinseed, feeding virtually ceases (or is certainly diminished) at 2°C.

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SPATIAL AND TEMPORAL PATTERNS OF SPRINGTIME UTILIZATION OF THE POTOMAC ESTUARY BY FISH LARVAE

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ABSTRACT

Ichthyoplankton were collected weekly in the upper Potomac estuary during the springs of 1974, 1975, 1976, 1977, and in 1980. In addition data were obtained on water quality, hydrology, zooplankton abundances, fish spawning stocks, and juvenile fish distributions. Larval fish food habits were also examined. This paper describes the spatial and temporal distributions of larval fishes and discusses how these distributions reduce competition for available habitat and food resources of this upper estuarine system.

INTRODUCTION

The Potomac estuary, a subsystem of the Chesapeake bay, has been utilized for fisheries exploitation as well as for transportation and sewage disposal since colonial times. Heavy industrialization has never been a major feature of the tidal Potomac, although it may be in the near future. Industrial and domestic growth potential is high on the western side of the Chesapeake Bay system as water and space are abundant, and energy facilities, the third necessary ingredient for growth, are increasing. Accordingly, in 1970 a nuclear power plant was proposed at the location in the upper Potomac estuary that coincided with the known center of the Potomac striped bass spawning grounds. As part of an evaluation of the potential impact of this facility, the Maryland Department of Natural Resources, through its Power Plant Siting Program, supported extensive studies on the key economic and resident fish species of concern, the striped bass. Field studies began in 1974 and continued through 1977. Included were investigations of estuarine hydrology, striped bass spawning stock assessment as well as ichthyoplankton and juvenile stocks. Larval striped bass food habit studies were also completed in addition to routine water quality and zooplankton investigations. A number of publications have been released on various facets dealing with population dynamics of striped bass from the above efforts (Mihursky et al. in press; Polgar et al. 1976; Polgar 1977; Setzler et al. in press; Setzler-Hamilton et al. 1980a, 1981; Ulanowicz and Polgar 1980).

In 1980, as part of the NOAA, NMFS national program designed to understand the decline of striped bass stocks on the east coast, an opportunity was provided to undertake another ichthyoplankton study in a segment of the Potomac Estuary that complemented the 1974-1977 investigations. This paper, while developed coincidental to striped bass studies, will deal primarily with other associated ichthyoplankton species found in the 1974-1977 and 1980 studies of the upper Potomac estuary.

It is generally held that the more similar two species are in size, habitat choice and morphology, the higher the probability that they will compete with each other (Hespenheide 1973). The larvae of most fish species are more similar to each other in size and morphology than are the adults of the same species. While the inexperienced observer might confuse larval naked gobies with larval striped bass, no similar confusion would occur with the adults. It would seem then that one of the greatest potentials for competition among fish species may occur in the larval fish community. Our purpose here is to examine the spring and early summer larval fish communities in a selected segment of the Potomac estuary and to determine where competition is most likely to occur, and to identify what mechanisms may be operating to reduce competition.

MATERIALS AND METHODS

Figure 1 shows the portion of the Potomac River estuary under investigation. The original 1974 sampling design consisted of 12 cross-stream transects separated by 6 to 12 km longitudinally and extending from Colonial Beach, Virginia, river kilometer 69, to Washington, D.C., km 176. Additional transects were sampled in 1975 but were not repeated in other years. Extreme transects were not sampled every year. Details of the sampling methodology are presented in Setzler-Hamilton et al. (1981).

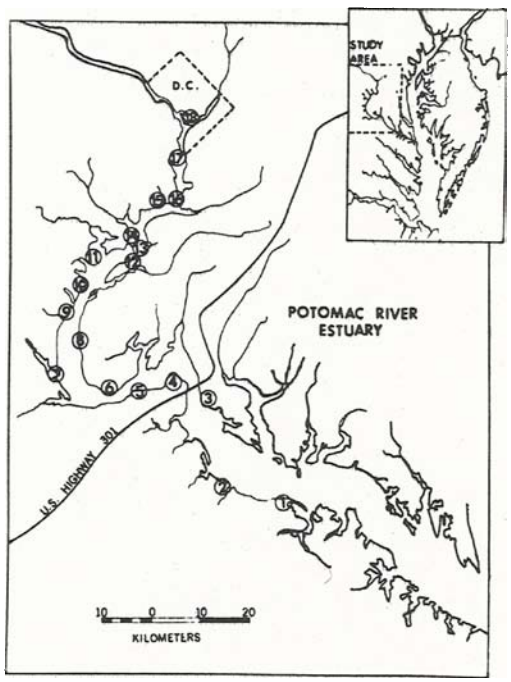

Figure 1. Potomac estuary showing areas sampled. Numbers indicate locations of sampling transects during 1974 to 1977 portion of study.

1 - river km 66.6
2 - river km 73.0
3 - river km 79.9
4 - river km 87.6
5 - river km 96.0
6 - river km 107.1
7 - river km 111.9
8 - river km 116.4
9 - river km 121.6
10 - river km 127.5
11 - river km 132.0
12 - river km 138.6
13 - river km 142.0
14 - river km 143.8
15 - river km 151.2
16 - river km 157.0
17 - river km 165.6
18 - river km 175.1

From 1974 through 1977 samples were collected at stations along fixed transects shown in Fig. 1 and Table 1. During 1980 we used a stratified random sampling regime for channel stations only, with transect locations 4, 5, 6, 8, 10, 12 and 14 (Fig. 1) serving as the mid-point of each sample region. Also the

cross-river location of each station was determined randomly within the channel region with the constraint that water depth was at least 6 m. We sampled weekly or more frequently during spring for the 5 years of these studies. Starting and ending dates and transects sampled during each year are summarized in Table 1.

Table 1. Sampling sites for the period 1974 to 1980.

Transect No.	River km	1974 4/1 8/19	1975 3/31 7/15	1976 3/30 6/23	1977 4/14 6/2	1980 4/15 6/3
1	68.6	X				
2	73.0	X				
3	79.9	X	X	X		
4	87.6	X	X	X		X
5	96.0	X	X	X	X	|
6	107.1	X	X	X	X	|
7	111.9		X			|
8	116.4	X	X	X	X	|
9	121.6		X			|
10	127.5	X	X	X	X	|
11	132.0		X			|
12	138.6	X	X	X	X	|
13	142.0		X			|
14	143.8	X	X	X	X	X
15	151.2		X			
16	157.0	X	X	X	X*	
17	165.0		X			
18	175.1	X	X			

* Only sampled on 5 May and 12 May in 1977.

Sampling was done with 1-m, 505 μm mesh monofilament nylon plankton nets equipped with calibrated General Oceanics flowmeters, Model 2030. For the period 1974 to 1977 each channel station (greater than 3-m in depth) was sampled with a stepwise oblique tow at 3-m depth intervals; each shoal station (≤ 3 m in depth) was sampled with a surface tow. During 1980 we sampled each station with surface, mid-depth, bottom and oblique ichthyoplankton tows. The surface samples were collected using a side arm to tow the net in the undisturbed water to the side of the vessel. Mid-depth tows were made using a 1-m plankton net dropped through the surface layer and retrieved through it as quickly as possible to minimize contamination. Oblique tows were made as in previous years; bottom tows were made with a Dovel plankton sled (Dovel 1964).

Oblique tows filtered from 125 to 500 m^3 during 5 years of sampling; horizontal tows sampled between 250 and 400 m^3. Only the oblique samples are used for the 1980 data when data were compared among years.

Samples were washed down into buckets and the contents poured through 505 μm mesh filters. These filters and the retained larvae were then placed in 10% buffered formalin. The formalin used in 1980 had rose bengal stain added to facilitate separation in the laboratory.

Water temperature, salinity and dissolved oxygen were measured at each station at 3-m depth intervals. In 1974 to 1977 light transmittance was also measured. Temperature and salinity were measured with a Beckman salinometer, Model No. RS5-3. Dissolved oxygen was measured with a YSI Model 54 oxygen meter. Transmittance was measured with a Beckman Model EV 4 Envirotron turbidity meter. In 1980 Secchi disc readings were used instead. Weather and tidal conditions were also noted.

In the laboratory the ichthyoplankton were sorted and larvae were identified to the lowest practical taxon. Centrarchids, clupeids and most cyprinids were not identified below family level. For this analysis, transect mean densities of ichthyoplankton were calculated as

$$\bar{D} = \left[\sum_{i=1}^{n} Ni / \sum_{i=1}^{n} Vi\right] \times 1000 \text{ (Pyne 1976)}$$

where Ni is the number of ichthyoplankton found in Vi m^3 of water strained in the i^{th} tow.

RESULTS

Table 2 lists the taxa encountered during these studies. Three taxa were abundant in all years: clupeids (*Alosa* spp. and *Dorosoma cepedianum*), white perch (*Morone americana*) and striped bass (*M. saxatilis*). Other species which were abundant in one or more years were the bay anchovy (*Anchoa mitchilli*), silversides (*Menidia* spp. and *Membras martinica*), yellow perch (*Perca flavescens*) and naked goby (*Gobiosoma bosc*). Since the above taxa comprised >99% of all ichthyoplankton sampled, our detailed analysis was limited to those forms.

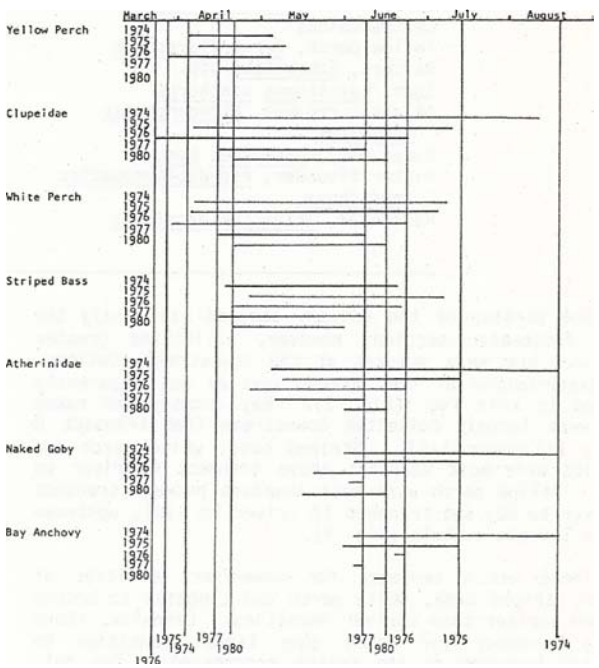

Figure 2. Seasonal presence of major larval taxa in the Potomac River estuary. Vertical lines represent beginning and ending of sampling periods.

Spawning in fishes is often associated with temperature increases. River temperature fluctuated from year to year and is reflected in the time of larval presence (Figure 2 and Table 3). Clupeid larvae first appeared in the middle of April in 1974 and earlier in 1975 and 1976 and might have appeared earlier in 1977 and 1980 if we had begun sampling sooner. Silversides larvae appearance fluctuated from the second half of April to about the second week in June. In 1974, 1975 and 1976 striped bass larvae were first present in the last quarter of April but they were already present when collections began on 14 April 1977 and 21 April 1980 so that time of first appearance is unknown for those years. Bay anchovy larvae appeared at the beginning of June in 1974, 1975, 1977 and 1980 but late in June in 1976.

Table 2. Larval fishes found within the study area and their relative abundance.

R = rare, found at fewer than 5 stations/year and/or densities always below 5 larvae/1000 m^3,

U = uncommon, found at fewer than 10 stations/year and/or densities always below 10 larvae/1000 m^3,

C = common, found at more than 10 stations/year and/or densities frequently above 50 larvae/1000 m^3,

A = abundant, found at more than 30 stations and/or with at least some stations with densities >500 larvae/1000 m^3.

	1974	1975	1976	1977	1980
Clupeidae (Alosa spp. or Dorosoma cepedianum	A	A	A	A	A
Bay anchovy, Anchoa mitchilli	A	C	R	R	R
Cyprinidae (except Carp)	-	R	R	R	C
Carp, Cyprinus carpio	U	U	-	R	-
Catfish, Ictalurus spp.	-	R	R	-	-
Cyprinodontidae	-	-	-	R	-
Silversides, Menidia spp. or Membras martinica	A	A	C	C	C
White perch, Morone americana	A	A	A	A	A
Striped bass, Morone saxatilis	A	A	A	A	A
Centrarchidae	U	R	U	R	-
Yellow perch, Perca flavescens	C	A	A	C	-
Darters, Etheostoma spp.	U	-	C	U	-
Spot, Leiostomus xanthurus	-	U	-	-	-
Atlantic croaker, Micropogonias undulatus	-	C	-	-	-
Naked goby, Gobiosoma bosc	A	A	A	R	R
Winter flounder, Pseudopleuronectes americanus	R	-	-	-	-
Hogchoker, Trinectes maculatus	R	-	-	-	C

The portion of the estuary sampled was mostly the tidal freshwater section; however, salinities greater than 0.5 ppt were present at the downstream stations. The distribution of the various species was apparently related to this fact (Fig. 3). Bay anchovy and naked goby were largely collected downstream from transect 8 (river kilometer 117). Striped bass, white perch and clupeids were most abundant above transect 6 (river km 107). Yellow perch were most abundant between transect 3 (river km 80) and transect 12 (river km 138), upstream of the 1.9 ppt isohale (Fig. 4).

There was a tendency for downriver densities of larval striped bass, white perch and clupeids to become reduced earlier than upriver densities. Likewise, there was a tendency for naked goby larval densities to increase upstream as the season progressed. The only year when sampling occurred sufficiently late for densities of bay anchovy to be analyzed was 1974. They appeared to mimic the pattern of the naked goby that year.

Vertical distribution of ichthyoplankton during daylight hours was obtained for 1980. During 1974 diel vertical migration patterns were obtained for white perch larvae. These diel patterns are used in assisting the interpretion of the 1980 data. Fig. 5 shows that tidal phase may influence diel patterns. It is also obvious from a comparison of Figure 5, Figure 6 and Table 3 that the vertical distribution of white perch over the whole 1980 collecting season more clearly resembles the pattern where the flood tide is at midnight. Differences in densities between depth strata were tested for the three abundant species of 1980 using Student's t test. Clupeid larvae had significantly higher densities in surface collections than at mid-depth ($t=2.454$, $df = 52$, $p<.02$) and the bottom ($t=4.760$, $df=52$, $p<.001$). White perch had their greatest densities at mid-depth. These collections showed significant differences with both surface collections ($t=5.170$, $df=52$, $p<.001$) and bottom samples ($t=2.18$, $df=52$, $p<.05$). Bottom samples showed significantly higher densities of white perch larvae than did surface samples ($t=4.70$, $df=52$, $p<.001$). Striped bass larvae occurred in significantly lower densities in the surface samples than they did in mid-depth collections ($t=2.870$, $df=12$, $p<.02$) or bottom samples ($t=2.468$, $df=18$, $p<.05$); however, differences between mid-depth and bottom striped bass densities were insignificant ($t=0.780$, $df=18$, $p>.05$).

DISCUSSION

Mean water temperatures and times of first occurrences of fish eggs and larvae of the species under consideration for the 5-year sampling period are summarized in Table 4. Environmental conditions associated with the spawning of these species, as surmised from the literature, are listed in Table 5. Initiation of spawning in yellow perch, white perch, striped bass, clupeids and silversides seems to be associated with temperature, whereas spawning of bay anchovies and naked gobies is apparently linked to intrusion of saline waters.

White perch eggs[1] were collected during the first cruise in all five years, though it is obvious from mean water temperatures and the presence of white perch larvae in collections for the years 1976, 1977 and 1980 that the spawn was already underway for those years. Likewise, the striped bass spawn was underway when we began sampling in 1977 and 1980. Although striped bass eggs were first collected during the last week of March at a mean water temperature of 12C in 1976, yolk-sac

1 Although white perch eggs are adhesive and demersal and were not quantitatively sampled, general spawning trends can be determined from our data.

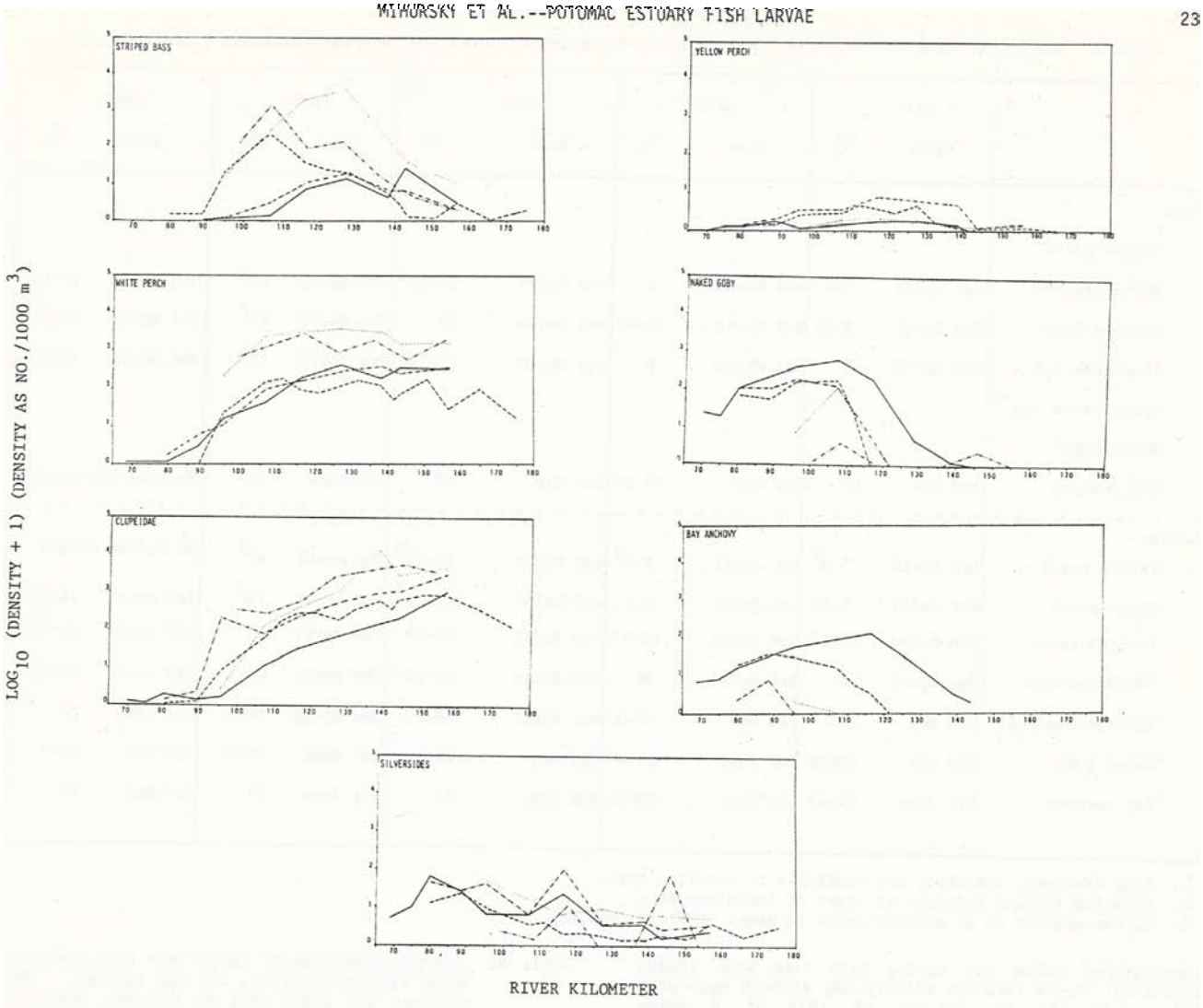

Figure 3. Distribution within the estuary of the seven principal taxa. Densities are expressed as Log_{10} of numbers of larvae/1000m^3.

_____ 1974 1977
__ __ 1975 __.. 1980
__. 1976

larvae were not found until a month later (Table 4). Densities of larval striped bass from the Potomac estuary were lower in 1976 than in 1975, 1977, and 1980 and survival to he finfold and later stages was poor (Setzler-Hamilton et al. 1981).

Peak densities of both white perch and clupeid larvae were witnessed further upstream as the spawning season progressed, which suggests a continual upstream migration of the adult spawners. Similar patterns were reported in the neighboring Patuxent estuary during 1978 and 1979 (Setzler et al. 1979; Mihursky et al. 1980).

Yellow perch larvae were collected from the beginning of sampling in 1974, 1976 and 1977, and from early April in 1976, until early to mid-May. In the Patuxent estuary yellow perch larvae were collected in fresh to oligohaline waters (maximum salinity of 5.0 ppt in 1978 and 4.4 ppt in 1979) from the first week of April to mid-June in 1978 and to mid-May in 1979 (Mihursky et al. 1980).

Although minimal spawning temperatures reported for silverside larvae range from 18-21C (Table 5), we collected silverside larvae at water temperatures of 14 to 17C. Either silverside spawning in the Potomac is initiated at lower temperatures than previously reported, or water temperatures along the shorelines and shallows where these fishes spawn (Martin and Drewry 1978) warmed more rapidly than the deeper waters where we collected their larvae.

The distribution of larval bay anchovies and naked gobies is related to river flow and to upriver intrusion of saline waters. In 1976 bay anchovy eggs were collected during the second week of May, their earliest appearance during the five years of sampling. The 3.9 ppt isohale progressed above Transect 3 at river km 80, (Figure 4) by mid-May of that year, the earliest of the 3-year period (1974-1976) during which sampling extended to Transect 3. Peak spawning of bay anchovies reputedly occurs at water temperatures above 20C (Table 5). Our data suggest that although bay anchovy eggs were taken at 13-14C in the Potomac estuary (Table 4), survival rates were such that no larvae were collected at water

Table 3. Mean Water Temperature, C and Time of First Occurrence of Fish Eggs and Larvae in the Potomac Estuary

	1974		1975		1976		1977		1980	
	Week	°C	Week	°C	Week	°C	Week	°C	Week	°C
Eggs										
Yellow perch[1]										
White perch	1st April	7-8	end March	7	end March	11-12[2]	2nd April	13[2]	3rd April	14-16[2]
Striped bass	2nd April	9-10	3rd April	11-12	end March	12	2nd April	13[2]	3rd April	14-16[2]
Clupeidae spp.	2nd April	10	end March	8	end March	11-12[2]	2nd April	13[2]	3rd April	14-16[2]
Atherinidae spp.[1]										
Naked Goby[1]										
Bay Anchovy	3rd May	19	4th May	13-14	2nd May	17	1st June	23	No eggs collected	
Larvae										
Yellow perch	1st April	7-8[3]	1st April	8-9[3]	end March	11-12[3]	2nd April	13[3]	No larvae caught	
White perch	2nd April	9-10	1st April	8-9	end March	11-12[3]	2nd April	13[3]	3rd April	14-16[3]
Striped bass	4th April	12-14	4th April	13-14	4th April	17-19	2nd April	13	3rd April	14-16[3]
Clupeidae spp.	2nd April	10	1st April	9	end March	11-12[3]	2nd April	13[3]	3rd April	14-16[3]
Atherinidae spp.	1st May	14	1st May	15-16	end April	16-17	2nd April	16-17	1st June	24
Naked goby	end May	19-20	1st June	23-24	3rd May	19-21	1st June	23-25	3rd May	19
Bay Anchovy	4th June	22-23	1st June	23-24	4th June	25	1st June	23	3rd May	20

1. Eggs demersal, attached; not available to sampling gear.
2. Spawning already underway at start of field sampling.
3. Larvae assumed to be present prior to start of field sampling.

temperatures below 20C during this five year study. Similarly, in the Patuxent estuary bay anchovy eggs were first collected in mid-May of 1979 at a water temperature of 21C, and in mid-June of 1978 at water temperatures of 23-24C; larvae were not collected until mid-June of both years at water temperatures of 23-24C (Setzler et al. 1979; Mihursky et al. 1980).

Naked gobies spawn in estuarine waters, and eggs are deposited on clam and oyster shells (Fritzsche 1978). Larvae move progressively upriver with the salt wedge; peak larval densities occurred at salinities of 1-5 ppt. Naked goby densities also peaked at salinities of 1-2 ppt in the Patuxent estuary during 1978 and 1979 (Setzler et al. 1979; Mihursky et al. 1980).

Engraulid and clupeid larvae apparently feed primarily on naupliar and copepodite stages of copepods (Schumann 1965, Berner 1959, Detwyler and Houde, 1970, Kjelson et al. 1975, Dekhnik et al. 1970, Duka 1969, June and Carlson 1971, Arthur 1977). Clupeid and engraulid larvae are collected in the upper layers of water (Ahlstrom 1959, Dovel 1971, Hempel and Weikert 1972) where nauplii and copepodites of copepods are most concentrated (Hempel and Weikert 1972, Tsyban' and Polishchuk 1969). Both Morone spp. and naked goby larvae tend to be deeper in the water column than clupeids (Martin and Setzler-Hamilton 1981). The diet of striped bass larvae is determined to be primarily adult copepods and cladocerans (Miller 1978; Beaven and Mihursky (1979) as is that of white perch (Setzler-Hamilton, Mihursky, Drewry and Martin, Chesapeake Biological Laboratory, Solomons, Maryland 20688, unpublished data). The diet of naked goby larvae is unknown, but larvae of other goby species in the

Table 4. Average densities of larvae for 1980 discrete depth samples (surface, mid and bottom). The averages are based only on stations where at least one sample of the three depths contained larvae of that species. Densities are expressed as number of larvae/1000m^3.

	Surface $\bar{X}$ (Range)	Mid-depth $\bar{X}$ (Range)	Bottom $\bar{X}$ (Range)
Clupeidae	4791.70 (0-56,040	2239.67 (0-32,801)	737.51 (0-17,311)
White perch	239.09 (0-3890)	3571.37 (0-31,460)	1957.31 (0-29,048)
Striped bass	21.58 (0-156)	239.53 (4-1012)	327.95 (4-2145)

Black Sea are reportedly opportunistic feeders, feeding on up to 20 species of plankton organisms (Dekhnik et al. 1970). Clupeid larvae found in the Potomac are known to tolerate brackish water (Jones et al. 1978, Dovel 1971) as can the Morone spp. (Hardy 1978, Dovel, 1971). We suggest that as larvae the bay anchovy and Alosa spp. are ecological equivalents as are the naked goby and the Morone spp., the slight disparity, in time and space, in their occurrence as larvae results from differences in spawning season and salinity preference, and are possible mechanisms whereby competition between

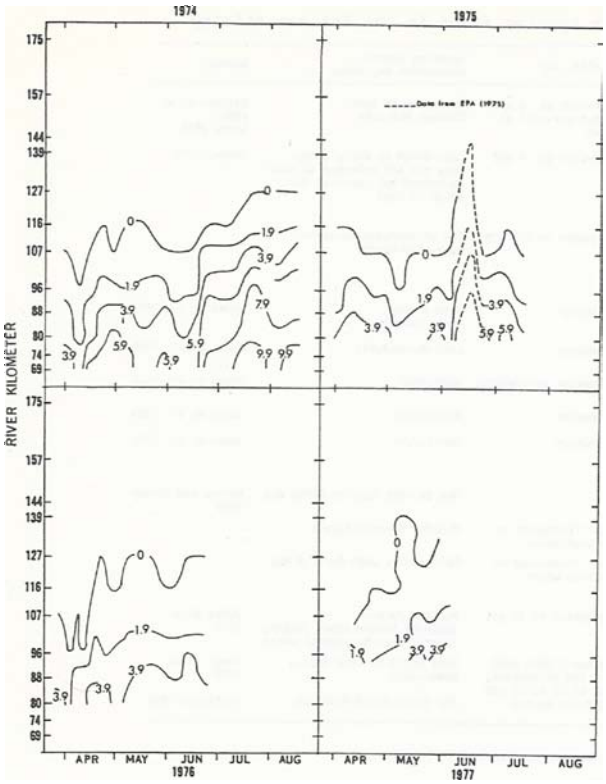

Fig. 4. Mean water column salinity, ppt, Potomac estuary; 1974-1977.

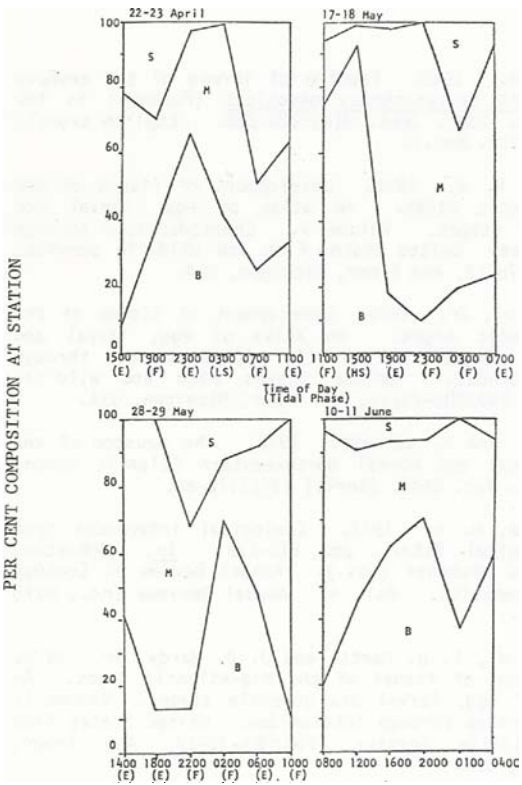

Figure 5. Diel depth distribution of white perch larvae on four dates in 1974. S = surface; M = mid-depth (4 m); B = bottom; E = ebb tide; F = flood tide; LS = low slack condition; HS = high slack condition.

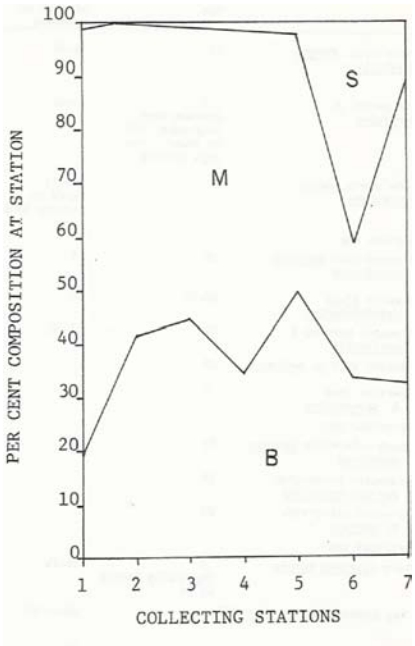

Figure 6. Depth composition of 1980 white perch larvae collections averaged over the whole collecting period. Station 1 is the most downstream station and 7 the most upstream. S = surface; M = mid-depth; B = bottom.

Note: 1980 collecting stations given here do not coincide with 1974-79 transect numbers, since 1980 collections were made with a stratified random sampling design.

ecological equivalents is reduced. Such reduction in competetion for food would appear to have special value in these upper estuarine segments as year to year variations in zooplankton densities are known to exceed a ten-fold difference (Setzler-Hamilton et al. 1981).

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Table 5. Environmental conditions for spawning of abundant fish species found in the Potomac estuary.

	Min	Water temp °C at peak of spawning	Max	Salinity, ppt	Spawning season Chesapeake Bay Areas	Source
Striped bass, Morone saxatilis	11	14-19	23	Freshwater to 3 ppt maximum survival at =1 ppt	Beginning of April Through mid-June	Setzler et al 1980 Hardy 1978
White perch, M americana	1 2 minimum temp tolerance, 10° or lower - few eggs survive	11-16	20 (eggs have been incubated from 21 to 25°C)	Freshwater to 4 ppt	Late March to early June-- Eggs not all released at once ovulation may continue for 10 to 21 days	Hardy, 1978
Yellow perch, Perca flavescens	5	B 5-11 based on surface temp	13	Fresnwater to 2 5 ppt	End of February to April, peak--Mid-March	
Clupeidae spp						
Gizzard shad Dorosoma cepedianum	10	18	29	Freshwater	Late spring, early summer	Jones et al 1978
Alewife Alosa pseudoharengus	10 52	18	29-31	Freshwater	Late March-April	Jones et al 1978
Blueback herring A aestivalis	14	21-26	27	Freshwater to brackish	April-May	Jones et al 1978
Hickory shad A mediocris	15		31	Freshwater	April-June	Jones et al 1978
American shad A sapidissima	8	18	26[3]	Freshwater	April-July	Jones et al 1978
Atherinidae spp						
Rough silverside Membras martinica	21	31	5 to 25 ppt		May to late July or early Aug	Martin and Drewry 1978
Tidewater silverside Menidia beryllina	18		30	Tidal freshwater or brackish water	Mid-May through August	
Atlantic silverside M menidia	20		30	Tidal freshwater or brackish water	March-July, peak April & May	
Cyprinidae spp						
Carp Cyprinus carpio	9 (typically begins at 14	18-25	32 (usually ceases above 29)	Freshwater to 10 ppt	May and June-- spawning intermittent lasting several days to several weeks	Jones et al 1978
Bay anchovy, Anchoa mitchilli	9	above 20	31	Minimum 1 ppt, most at 9 ppt or greater, peak at 13 to 15 ppt	Late April to late Sept , peak--July	Jones et al 1978
Naked goby, Gobiosoma bosc	19	ND	29	Estuarine waters	May through mid-November	Fritzsche 1978

1 Alewife spawning generally precedes blueback spawning by 3 to 4 weeks, spawning peaks separated by 2 to 3 weeks

2 Minimum spawning temperature for alewife 7.0°C, but 70% deformed larvae below 10 6

3 American shad spawning generally occurs at 12 to 21

D = No Data

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ICHTHYOPLANKTON DENSITY FLUCTUATIONS IN THE LOWER SUSQUEHANNA RIVER, PENNSYLVANIA, FROM 1976 THROUGH 1980

Barbara F. Lathrop

Abstract.--Ichthyoplankton sampling was conducted in York Haven Pond, a lower Susquehanna River impoundment near Harrisburg, Pennsylvania, to study occurrence, distribution, abundance, and composition of fish larvae. Sampling was done weekly at night along shore with pushed 0.5 m nets (1976 and 1977) and later expanded to include day sampling (1978 through 1980). Seven species, members of Cyprinidae, Catostomidae, Centrarchidae, and Percidae, comprised about 90% of the catch each year. Large diel differences were found; 80 to 85% of the annual catch was taken at night. Some species, notably the ictalurids, were taken almost exclusively at night. Significant variation in abundance due to station, date, and year effects was indicated by analysis of variance. However, cluster analyses showed station and community similarities based on species composition of the seasonal catch. Multiple stepwise regression analyses showed that day length, sunlight, and various temperature parameters were important factors in explaining density variations of ichthyoplankton populations.

INTRODUCTION

Ichthyoplankton sampling is an integral part of many environmental studies associated with power plant construction and operation. In the past decade, these studies have revealed much new information concerning ichthyoplankton populations and their dynamics. This study was part of the ecological research for the Three Mile Island Nuclear Station and is presented to contribute to the knowledge of spatial and temporal abundance and composition of ichthyoplankton populations.

STUDY AREA

York Haven Pond is a 760 hectare mainstream impoundment on the Susquehanna River in south-central Pennsylvania, 16.1 km (10 miles) southeast of Harrisburg. The impoundment was created to serve York Haven Hydroelectric Plant. The reservoir also serves the cooling water needs of Three Mile Island Nuclear Station (TMINS) and supports recreational fishing and boating.

TMINS is located on the largest of several islands in the reservoir; two intakes and a main discharge situated on the west shore of Three Mile Island (TMI) utilize the center channel of the river (Fig. 1). This channel receives 24 to 30% of the total river flow (Gilbert Associates 1979). The east channel is non-flowing during periods of low river discharge (summer flows $\leq$ 566 m^3/s or 20,000 cfs) due to greater height of the east dam. The west channel carries the remaining flow year-round.

METHODS

In 1974 and 1975 entrainment studies at the TMINS and related larval fish samples revealed that peak ichthyoplankton abundance occurred after sunset (Potter and Associates 1975, 1976). Farfield sampling in 1975 showed that larvae of most species were more abundant nearshore rather than in midchannel (Lathrop 1976, Ritson 1977). With this information, the present program was initiated in 1976.

Ichthyoplankton was sampled weekly at 14 stations (13 in 1976) throughout the reservoir from April through August. Stations were sampled in random order each date to minimize the bias of time of day (Lathrop 1979). Only night (after sunset) samples were taken in 1976 and 1977; from 1978 through 1980, day (after 0800 h) and night samples were taken about 12 hours apart each week. Replicate surface samples were taken at each station with paired 0.5 m conical plankton nets (0.5 mm mesh) fitted to square frames set off the front of a boat. The boat was powered upstream at 10 to 20 m offshore for four minutes, sampling about 200 m of shoreline. Water depth at most stations was about 1.0 m. Water volume filtered was estimated with a General Oceanics flowmeter mounted in the center of each net mouth. Samples were preserved with 20 to 25% formalin.

Larvae were identified to the lowest feasible taxon, measured, and enumerated. Pumpkinseed and bluegill sunfish larvae were non-separable and were enumerated as one category, Lepomis gibbosus/L. macrochirus. Catch rate was expressed as number of larvae (n) per 100 m^3 of water. Statistical tests were run on log-transformed densities, $\log_{10}(n/100\ m^3 + 1)$ to help linearize and normalize the data (Sokal and Rohlf 1969). Some analyses were limited to night samples because fewer zeros and larger numbers provided a more robust data set. Computed ratios were simply night numbers (or density) divided by day numbers (or density) (N/D).

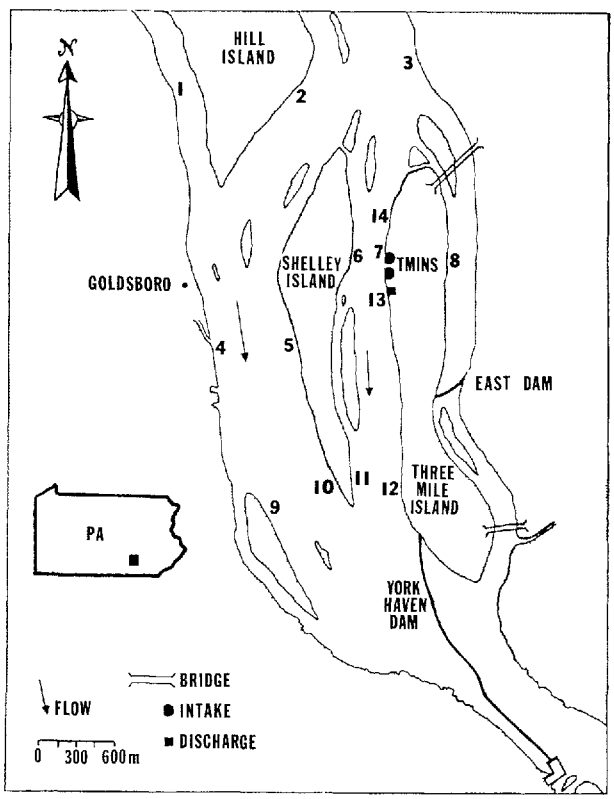

Figure 1. Locations of Ichthyoplankton stations (0.5 m push net) sampled in York Haven Pond 1976 through 1980.

Three- and four-factor analyses of variance (ANOVA) were used to ascertain the effects of years, stations, dates, and replicates (Sokal and Rohlf 1969). The Student-Newman-Kuels multi-range test (SNK) was applied to identify means not significantly different (Woolf 1968). The 95% confidence level ($P \leq 0.05$) was utilized unless otherwise noted.

Day - night density relationships were investigated using the pooled catch for all 14 stations for day and night data to determine the degree of rank correlation for the day and night catches within and between the years (1978 through 1980). For the regressions, night values were used as the independent variable to predict day densities, the dependent variable. The intent was to investigate if elimination of day sampling was feasible since the night catch yielded more larvae per effort and provided a better data set for the study of annual variations in abundance. Transformed densities for the

14 stations were pooled (summed each date) to reflect the densities of the entire reservoir. This decreased the inherent variability caused by station differences, and aided in the assessment of general trends in the reservoir.

Stepwise multiple linear regressions (MLR) were used to investigate the relationship between certain environmental parameters (independent variables) and ichthyoplankton density (dependent variable). Spawning in fishes is known to be related to increasing temperatures and daylight (Hynes 1970, Scott and Crossman 1973). These parameters were entered into the regression as heating degree days, cumulative river degrees, day length, and incident solar radiation to try to produce numerical indexes of springtime warming trends. Initially, 58 independent variables were tested to indicate which factors might be related to ichthyoplankton density (Lathrop 1980). Some variables were accumulated for 1, 2, 3 and 4 week intervals, or for 2 week intervals ending 1, 2, 3 or 4 weeks prior to sampling to encompass the probable spawning and hatching activities of fishes. Various base temperatures (34, 40, 50, and 68° F) were used to test for "trigger" temperatures. Data collected in the field, such as in situ air and water temperature, dissolved oxygen, pH, water clarity (secchi disc), time of day and current speed were also included.

Regressions on 1980 data were refined. Only those variables of some importance in previous regressions were used and sample size was increased by summing individual station densities each date.

Percent similarity (PSc) values based on percent composition of species were computed after Whittaker and Fairbanks (1958) to show composition similarities between stations and between years. These values were then used for construction of simple Bray-Curtis ordinations (Poole 1974) to investigate the community relationships among the stations. This results in a graphical clustering of stations with similar populations.

RESULTS AND DISCUSSION

The ANOVA tests on the ichthyoplankton data always indicated significant date, station, year and interaction effects (Lathrop 1978, 1979, 1980). Replicate and replicate interaction effects were not significantly different, meaning no sampling bias was associated with an individual net. F values for station and date effects were in the order of 200 to 500 with date being the most significant effect each year. This indicated the highly variable nature of the data and that season has a large influence on ichthyoplankton populations. However, variability among stations due to different physical and habitat characteristics, and among dates due to differences in species spawning times and abundances, would be expected. Despite these differences, consistencies were seen in species composition, seasonal occurrence, spatial and diel distributions, and in density relationships with certain environmental parameters.

Species composition.--Some 16,000 to 24,000 larval (about 95% of total) and juvenile fish (all under 25 mm TL) representing 20 to 32 taxa were taken each year. Common carp (Cyprinus carpio), spottail shiner (Notropis hudsonius), spotfin shiner (N. spilopterus), quillback (Carpiodes cyprinus), pumpkinseed/bluegill sunfish (Lepomis gibbosus/L. macrochirus), tessellated darter (Etheostoma olmstedi), and banded darter (E. zonale) were the most common taxa taken, comprising 88 to 94% of the total catch each year (Table 1). Ictalurids, chiefly the channel catfish (Ictalurus punctatus), a common adult fish in the reservoir, comprised less than 5% of the total annual catch. This was in part due to the sampling effort being concentrated nearshore; young ictalurids are more numerous (up to 20% of the composition) in midchannel drift (Lathrop 1976, Ritson 1977).

Table 1. Summary (number, n and percent, %) of the most common ichthyoplankton taxa taken during the day (D) and at night (N) in York Haven Pond 1976-1980. Day sampling was initiated in 1978. T denotes total day and night values.

		1976		1977		1978		1979		1980	
		n	%	n	%	n	%	n	%	n	%
Cyprinus carpio	D	-	-	-	-	1313	37.25	350	9.10	1142	26.47
	N	2367	12.46	5227	31.49	6903	33.16	4281	23.93	3505	20.04
	T					8216	33.76	4631	21.30	4647	21.32
Notropis hudsonius	D	-	-	-	-	265	7.52	101	2.63	54	1.25
	N	1847	9.72	1946	11.72	2875	13.81	1628	9.10	812	4.64
	T					3140	12.90	1729	7.95	866	3.97
Notropis spilopterus	D	-	-	-	-	99	2.81	304	7.90	128	2.97
	N	3865	20.34	1203	7.25	879	4.22	829	4.63	1537	8.79
	T					978	4.02	1133	5.21	1665	7.64
Total Cyprinidae	D	-	-	-	-	1697	48.14	798	20.74	1363	31.59
	N	8509	44.78	8591	51.75	10776	51.77	6863	38.36	5921	33.87
	T					12472	51.24	7661	35.24	7284	33.41
Carpiodes cyprinus	D	-	-	-	-	271	7.69	196	5.09	129	2.99
	N	3486	18.34	1361	8.20	4547	21.84	3729	20.84	2575	14.72
	T					4818	19.79	3925	18.05	2704	12.40
Total Catostomidae	D	-	-	-	-	375	10.64	638	16.58	159	3.68
	N	3951	20.79	2166	13.05	4849	23.30	4203	23.49	2784	15.91
	T					5224	21.46	4841	22.27	2943	13.49
Lepomis gibbosus/macrochirus	D	-	-	-	-	525	14.89	2117	55.03	2263	52.46
	N	2870	15.11	4240	25.54	2003	9.61	4172	23.32	6026	34.46
	T					2528	10.38	6289	28.93	8289	38.02
Total Centrarchidae	D	-	-	-	-	535	15.18	2130	55.37	2289	53.06
	N	3625	19.08	4357	26.25	2245	10.79	4397	24.57	6146	35.14
	T					2780	11.42	6527	30.02	8435	38.69
Etheostoma olmstedi	D	-	-	-	-	4	0.11	17	0.44	25	0.58
	N	772	4.06	666	4.01	276	1.33	1371	7.66	1274	7.28
	T					280	1.15	1388	6.38	1299	5.96
Etheostoma zonale	D	-	-	-	-	901	25.56	237	6.16	389	9.02
	N	1469	7.73	243	1.46	1653	7.94	662	3.70	760	4.35
	T					2554	10.49	899	4.14	1149	5.27
Total Percidae	D	-	-	-	-	916	25.99	281	7.30	486	11.27
	N	2605	13.71	1080	6.50	1968	9.45	2111	11.80	2196	12.56
	T					2884	11.85	2392	11.00	2682	12.29
GRAND TOTAL	D	-	-	-	-	3525		3847		4314	
	N	19003		16601		20815		17893		17487	
	T					24340		21740		21801	
No. of Taxa	D	-		-		24		23		20	
	N	29		30		26		29		30	
	T	29		30		32		30		30	

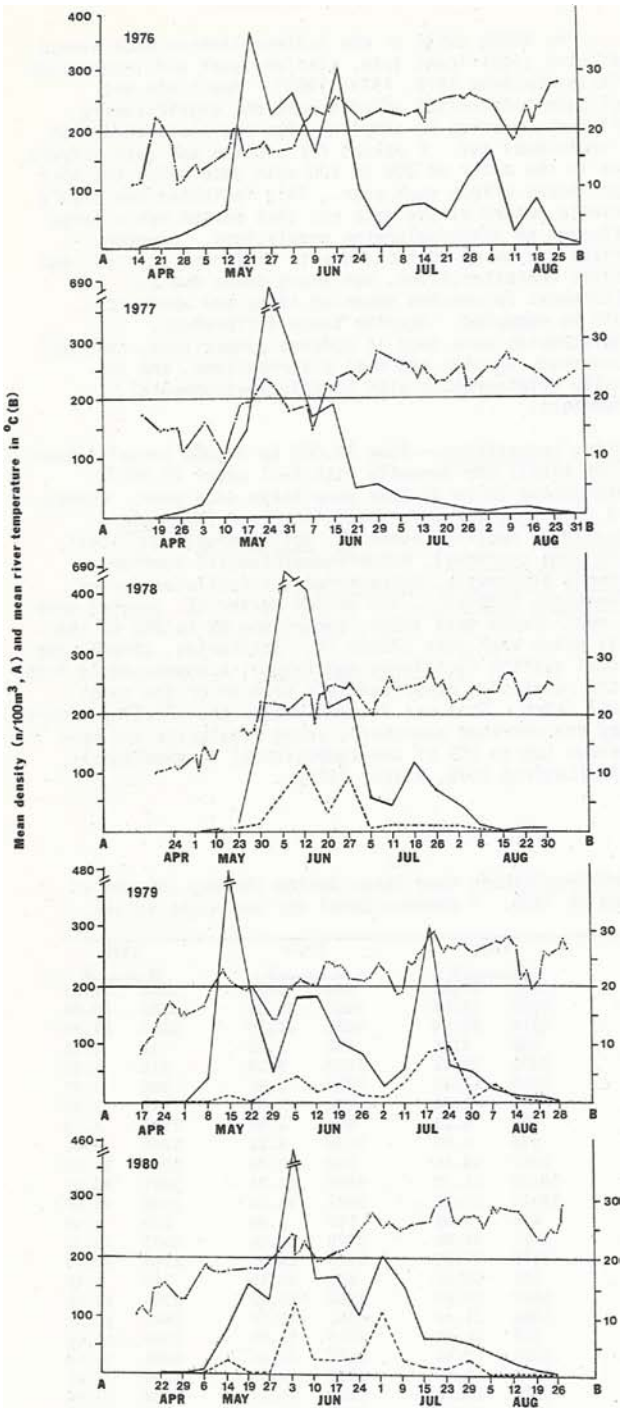

Figure 2. Mean density of ichthyoplankton (n/100 m^3, A) and mean river temperature (°C, B) recorded in York Haven Pond, 1976 through 1980. Dashed line (1978 through 1980) is day densities. The 20° C line is drawn for reference. Data between 10 and 23 May 1978 were not collected due to high river flows.

Seasonal distribution.--The seasonal patterns of population densities for night samples were generally similar. Densities increased through May, and peaked between mid-May and early June, just after mean river temperature reached 20 C (Fig. 2). Densities generally declined through August, with one or two secondary peaks usually occurring in July. High river flow (≥ 1133 m^3/s or 40,000 cfs) during the spawning season usually decreased densities or delayed the peak. Day densities generally followed the night patterns each year, but year to year trends and similarities were not as apparent in the day graphs (Fig. 2).

Only a few larvae were taken in April. The first larvae collected in the season were the white sucker (Catostomus commersoni), shield darter (Percina peltata), and walleye (Stizostedion v. vitreum) (Lathrop 1978, 1979, 1980). In early May, spottail shiner, quillback, tessellated darter, and banded darter were taken. The initial density peak was comprised mostly of carp, spottail shiner, and quillback larvae. By late May and early June, the shorthead redhorse (Moxostoma macrolepidotum) and northern hog sucker (Hypentelium nigricans) were taken, as well as the first centrarchids (smallmouth bass, Micropterus dolomieui, and pumpkinseed/bluegill). By late June, the carp, quillback, and spottail shiner larvae decreased in number, and the spotfin shiner, channel catfish, rock bass (Ambloplites rupestris), redbreast sunfish (Lepomis auritus), smallmouth bass, pumpkinseed/bluegill and crappies (Pomoxis spp.) were present. The secondary peak in July was comprised mostly of pumpkinseed/bluegill larvae. Carp and the suckers were virtually absent. By August, most species declined and spotfin shiner were predominant. The darters seemed to have the least defined spawning season, as they were present from May through July with most taken in June. Walleye were always most abundant early in the season (late April, early May) and absent after early June.

Spatial distribution.--Densities at the 14 stations were significantly different each year (ANOVA). Stations 1 and 8, both slack water areas by midsummer, yielded the most larvae each year; station 10 also yielded high catches (Fig. 3). SNK tests showed that these stations had significantly higher densities compared to other stations, and in most years were not significantly different from each other (Lathrop 1979, 1980). Stations 2 and 9, located near the upstream shores of islands with little upstream spawning habitat, had significantly lower densities of larvae. Station 6, adjacent to a steep bank, received mostly midchannel flows, and also yielded low numbers of larvae. All other stations were situated downstream from extensive shorelines where spawning could occur, and yielded intermediate numbers of larvae.

Although population densities among the stations were highly variable within each year, similarities were evident in species composition. Ordination of stations based on percent composition showed that stations 1 and 8 tended to be farthest removed from the other clusters of stations (because of low PSc values), and the west shore TMI stations were always closely clustered (highest PSc values) (Fig. 4). Stations usually clustered in agreement with their geographic location in the reservoir, i.e. west channel stations were closer to each other than to other stations, and center channel stations were clustered in a group. Stations 1 and 8 produced the highest densities of sunfish larvae, which made them unique in the graphics. Tightly grouped TMI stations were closely related in species composition probably because the flow along the west side of TMI tended to be laminar and carried the drifting larvae more or less as a unit.

Diel distribution.--The catch was notably higher at night for all major, and most other, taxa (Table 1). From 80 to 85% of the total catch each year was taken at night. Overall night/day ratios (N/D) were 5.9, 4.7, and 4.1 for 1978 through 1980, respectively. Individual collection ratios for each station each date were almost always higher at night; N/D was as high as 668. During the three years, only about 5 to 7% of the paired samples showed greater day catches. More taxa were taken in night samples each year (Table 1) and also on individual dates (Lathrop 1978, 1979, 1980). The catfishes were taken almost exclusively at night (95 to 100%). The most common species were best represented with higher densities in night samples. Most species comprised similar proportions, with respect to the total catch, in both day and night samples. The overall species

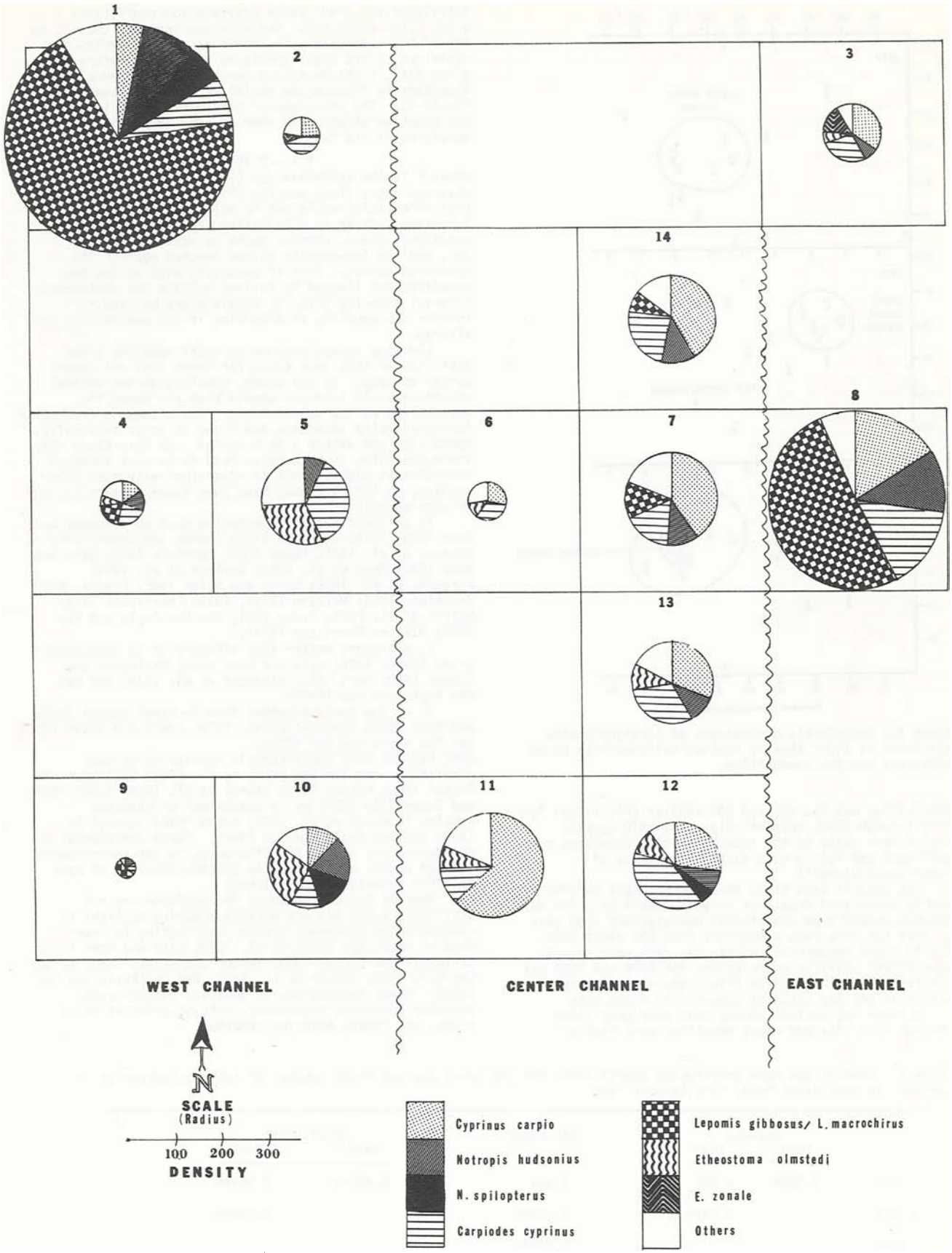

Figure 3. Mean density (n/100 m^3) and percent composition of ichthyoplankton taken at night at 14 stations in York Haven Pond, 1979. Stations are geographically oriented.

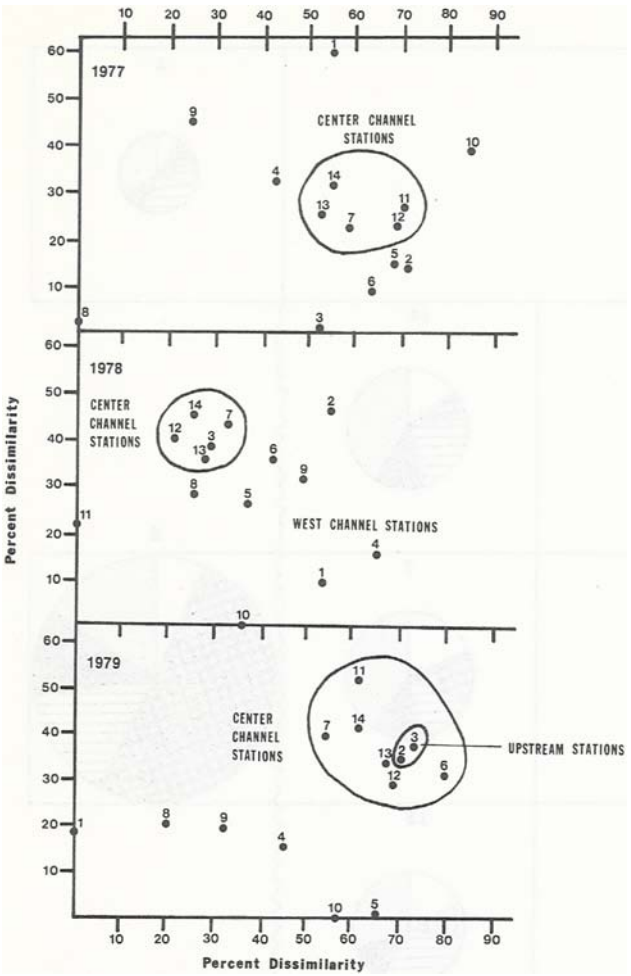

Figure 4. Bray-Curtis ordinations of ichthyoplankton data taken at night showing station relationships based on percent species composition.

composition was 71, 52, and 69% similar (PSc value) for 1978 through 1980, respectively. The 1979 samples varied more owing to the reduced daytime abundance of quillback and fairly high daytime abundance of pumpkinseed/bluegill.

The Kendall taus tests showed that night catches were highly correlated from year to year (Table 2). The day catches showed some significant correlations from year to year but were less concordant than the night data. Day to night comparisons within the same year showed significant positive correlations for 1979 and 1980 but not for 1978. These tests illustrate the more variable nature of the day catch as compared to night data.

Linear regressions showed that each year (1978 through 1980) day and night densities were highly correlated and predictable (correlation coefficient = 0.92, 0.90, and 0.85). These values indicate that 72 to 84% of the variability in densities of day samples was explained by the night densities alone. Therefore on any given date, night densities can be used to predict day densities by fitting the variables into the equations (Table 3). The three years' data were pooled to obtain one equation which could then be used to predict day densities in the future:

$$\hat{Y} = -0.75 + 1.002(x)$$

where $\hat{Y}$ is the estimated day (log) density and x is the observed night (log) density (Fig. 5). Application of this information would cut by half the sampling and laboratory effort by eliminating day sampling. On occasional dates, samples could be taken during the day, and the log-density values checked against the predicted values. Only if unusually high or low day densities are flagged by falling outside the confidence interval expected (Fig. 6) should there be need for further day sampling to determine if the populations have altered.

Limiting investigations to night sampling seems justified in this case since far fewer fish are caught during the day. In any study, sampling during optimal abundance would increase sample size and hence the reliability of the observations. Variations in freshwater ichthyoplankton abundance are known to occur seasonally, spatially, and within a 24 h period. We have found the ichthyoplankton in York Haven Pond to be most abundant nearshore at night. This is consistent with most other studies, as larval fishes have been found, generally, to be more abundant:

1) at night rather than during daylight (Buynak and Mohr 1976, 1977; Clifford 1972; Conner and Bryan 1976; Edwards et al. 1977; Faber 1967; Ferraris 1973; Gale and Mohr 1978; Geen et al. 1966; Gerlach et al. 1974; Kindschi et al. 1979; Lewis and Siler 1980; Lindsay and Northcote 1963; Molzahn 1973a, 1973b; Morrisson 1975; Netsch et al. 1971; Taber 1969; Van Den Avyle and Fox 1980; Wik and Morrisson 1974);

2) nearshore rather than offshore or in midchannel sites (Faber 1970; Gale and Mohr 1978; Gallagher and Conner 1980; Hart 1930; Kindschi et al. 1979; and Van Den Avyle and Fox 1980);

3) at the surface rather than in other depths (Gale and Mohr 1978; Kindschi et al. 1979; Lewis and Siler 1980; Van Den Avyle and Fox 1980).

Some studies have found certain species to be more abundant during the day (Cada et al. 1980; Gallagher and Conner 1980; Graser 1979; Storck et al. 1978; Taber 1969; and Tuberville 1979) or in midchannel or midwater samples (Edwards et al. 1977; Hatch 1980; Tuberville 1979; Van Den Avyle and Fox 1980). These differences in abundances may be due to differences in the environments sampled and/or differences in species-specific or age-specific behavior of the fishes.

Reasons for diel changes in abundance are not positively known but net avoidance during daylight is considered an important factor contributing to lower daytime densities (Cada et al. 1980; Gale and Mohr 1978; Gallagher and Conner 1980; Mathur and Heisey 1980; Nelson and Cole 1975; Netsch et al. 1971; Van Den Avyle and Fox 1980). Other factors may be negative phototropism, predator avoidance techniques, loss of orientation at night, and innate drifting behavior.

Table 2. Kendall tau rank correlation coefficients for the total day and night catches of ichthyoplankton at 14 stations in York Haven Pond, 1978 through 1980.

	DAY:DAY		DAY:NIGHT	NIGHT:NIGHT	
	1979	1980		1979	1980
1978	0.352*	0.209	0.341	0.681**	0.659**
1979		0.593*	0.615**		0.769**
1980			0.791**		

* Positively correlated at $P \leq 0.05$
** Positively correlated at $P \leq 0.01$

Table 3. Regression results and equations for predicting day densities of ichthyoplankton (Y) from night data (x) taken in York Haven Pond, 1978 through 1980.

	1978	1979	1980
Regression coefficient (slope, b)	1.20	0.87	1.60
Intercept (a)	-1.43	-0.28	-2.80
Equation*	$\hat{Y} = -1.43 + 1.2(x)$	$\hat{Y} = -0.28 + 0.87(x)$	$\hat{Y} = -2.8 + 1.6(x)$
Standard error (reg. coef.)	0.13	0.10	0.26
F	79.99	83.04	38.31
df	16	20	16
r	0.92	0.90	0.85
r^2	0.84	0.81	0.72

	1978 - 1980
Regression coefficient	1.00
Intercept (a)	-0.75
Equation*	$\hat{Y} = -0.75 + 1.002(x)$
Standard error (reg. coef.)	0.08
F	171.67
df	54
r_2	0.87
r^2	0.76

* $\hat{Y}$ and x in log values: $\log_{10}$(density + 1) where density = n/100m^3

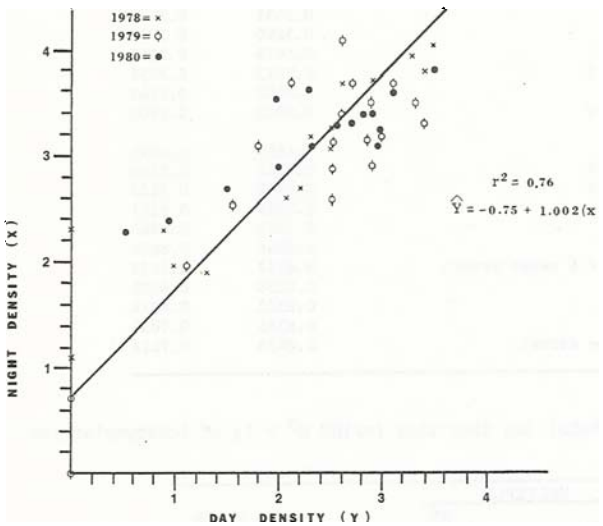

Figure 5. Relationship of observed night vs. observed day density [$\log_{10}$ (n/100 m^3 + 1)] of ichthyoplankton taken in York Haven Pond, 1978 through 1980. The equation for predicting day densities based on this relationship is given.

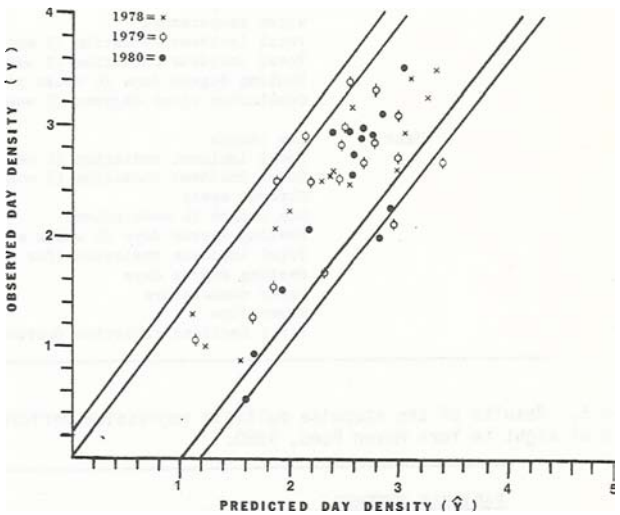

Figure 6. Comparison of the observed vs. predicted ichthyoplankton densities [$\log_{10}$ (n/100 m^3 + 1)] in York Haven Pond, 1978 through 1980. The 90 and 95% confidence intervals are shown.

Environmental factors affecting density.--Stepwise multiple regressions with 58 independent variables revealed that day length and heating degree days were important in explaining variability in population density of ichthyoplankton. Other variables also entered into the model such as incident solar radiation and lagged indexes of temperature and solar radiation (Table 4). Final r^2 values were 0.52, 0.74, and 0.71 for 1977 through 1979 data respectively, but the equations included 5 to 11 variables. This was cumbersome and unrealistic for a workable model, but it does indicate that day length and warming trends can be identified in a relationship with density fluctuations. MLR's with day densities yielded poor r^2 values: 0.57 and 0.35 for 1978 and 1979; however, many of the same variables were identified (Table 4). In 1978, time of day also proved to be a significant factor with a partial correlation coefficient of − 0.14 for 540 df, indicating that as time increased, density decreased. This substantiates the need for a randomized sampling regime. Ichthyoplankton densities had little or no linear relationship with field measured parameters.

It was interesting to note that for some species (carp, quillback, pumpkinseed/bluegill, and tessellated darter) moon phase was an important factor (Lathrop 1980). Correlation coefficients were − 0.30, − 0.22, 0.12, and − 0.18, respectively; carp, sucker and darter densities were higher during the darker phases. This may have been the result of increased net avoidance due to the available light on moonlit nights. Otherwise, these species densities were affected by most of the same variables as influenced overall densities (Lathrop 1980).

The regressions performed on 1980 night densities revealed relationships between ichthyoplankton and three environmental parameters--day length, heating degree days, and cumulative river degrees (Table 5). These three variables explained 95% of the variability in 1980 ichthyoplankton data with a correlation coefficient of 0.976. The correlation was much higher than with previous regressions, probably due to the use of one y variable for each date instead of 14. General climatological parameters that affected all stations in the same way environmentally were more related to general density trends of ichthyoplankton in the reservoir than were station parameters related to station densities. Future modelling with environmental parameters may be possible if this approach yields consistent relationships for all years. Combining similar stations may yield better models as well. In this way, predictable density variations in the ichthyoplankton could be monitored.

Table 4. Variation (r^2) of 1977 through 1979 day and night ichthyoplankton densities (log-transformed) explained by significant ($P \leq 0.05$) independent variables in stepwise multiple regressions.

Year	Phase	Variable entered	Multiple r	Multiple r^2
1977	Night	Day length	0.6320	0.3995
		Heating degree days	0.7084	0.5018
		D'ssolved oxygen	0.7176	0.5150
		pH	0.7205	0.5192
		Time	0.7234	0.5233
		Air temperature	0.7245	0.5249
1978	Day	Day length	0.6559	0.4302
		Total incident radiation (4 weeks prior)	0.6953	0.4834
		Total incident radiation (2 weeks prior)	0.7191	0.5171
		Air temperature	0.7303	0.5333
		Day length (2 weeks prior)	0.7476	0.5589
		Time of sample	0.7534	0.5676
	Night	Net heating/cooling degrees in river	0.7412	0.5494
		Heating degree days	0.7894	0.6231
		Day length	0.8173	0.6680
		Flow	0.8513	0.7247
		Total incident radiation	0.8581	0.7363
		Current speed	0.8630	0.7447
1979	Day	Day length	0.4208	0.1771
		River flow	0.4589	0.2106
		Current speed	0.5031	0.2531
		Water temperature	0.5290	0.2798
		Total incident radiation (1 week prior)	0.5439	0.2958
		Total incident radiation (2 weeks prior)	0.5523	0.3051
		Heating degree days (4 weeks prior)	0.5607	0.3143
		Cumulative river degrees (2 weeks prior)	0.5922	0.3507
	Night	Day length	0.6855	0.4699
		Total incident radiation (4 weeks prior)	0.7141	0.5100
		Total incident radiation (3 weeks prior)	0.7198	0.5182
		Current speed	0.7239	0.5241
		Day length (1 week prior)	0.7310	0.5344
		Heating degree days (4 weeks prior)	0.8046	0.6474
		Total incident radiation (for 2 weeks at 4 weeks prior)	0.8137	0.6622
		Heating degree days	0.8258	0.6820
		Water temperature	0.8322	0.6926
		River flow	0.8385	0.7031
		Total incident radiation (between sample dates)	0.8436	0.7116

Table 5. Results of the stepwise multiple regression performed on total log densities ($n/100\ m^3 + 1$) of ichthyoplankton taken at night in York Haven Pond, 1980.

VARIABLE ENTERED	MULTIPLE R	MULTIPLE R^2	F TO ENTER
Day length (x1)	0.864	0.746	52.96
Heating degree days (x2)	0.959	0.919	36.17
Cumulative river degrees (x3)	0.976	0.952	10.86

Regression coefficient (x1)	0.198
Regression coefficient (x2)	-0.224
Regression coefficient (x3)	0.008
Intercept (a)	-40.811
Standard error (x1)	0.264
Standard error (x2)	0.068
Standard error (x3)	0.002
df	15

CONCLUSION

The ANOVA tests always indicated significant year to year differences in density as well as date and station differences within each year. Graphic and tabular presentations of the data attest to the inherent seasonal variation (date differences), station variation, and species abundance differences. However, overall species composition and abundance were similar each year. Moreover, similarities in species composition at individual stations were consistent throughout the years, as well as relative densities at each station. Major density peaks occurred each year when river temperature reached 20 C from mid-May to early June, and the overall density variations were similarly related to day length and temperature parameters.

Highly variable ichthyoplankton data can present problems when power plant effects are of interest. However, if underlying consistencies and trends in the data are ascertained, perhaps localized effects near the discharge or at downstream stations can be detected. It is desirable to determine the extent and type of natural variation within a data set for baseline comparisons in the future. Use of a variety of methods should help to view the data in different ways and provide indexes of population perturbations until more precise models are developed. The methods presented herein might be useful with other ichthyoplankton data. Day-night relationships may be predictable in many other studies once a two or three year data base is established. Modelling density relationships with environmental parameters may or may not be possible in

other environments, depending upon the correlation between the major species population dynamics and various light and temperature regimes.

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TEMPORAL AND SPATIAL DISTRIBUTION OF SOME YOUNG-OF-THE-YEAR FISHES IN DEGRAY LAKE, ARKANSAS, 1975-1978

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ABSTRACT

Midwater trawling in DeGray Lake and townet sampling in the reservoir discharge were used to describe annual, temporal and spatial distribution of young-of-the-year (YOY) fish from 1975 through 1978, shad (Dorosoma spp.), crappies (Pomoxis spp.), and sunfishes (Lepomis spp.) composed 98% of the YOY fish collected. Shad reached peak abundance during the last week of May or the first week of June each year. Crappies were most abundant in mid-May in midwater trawl collections in the lake, even though discharge sampling indicated that crappies peaked in early May in 1976 and 1977 just before midwater trawl sampling was started. Sunfishes were collected from May through July and were most abundant in June. High numbers of sunfishes in 1977 and lower densities of gizzard shad (D. cepedianum) in 1977 than in 1978 were discussed in light of possible competition for food. Relative densities of the three principal groups varied from year to year, but total biomass of YOY fish (shad, crappies, and sunfishes combined) remained relatively stable.

INTRODUCTION

Knowledge of spatial and temporal variations in abundance of young-of-the-year (YOY) fishes contributes to a better understanding of population dynamics of reservoir fishes and aids in developing management procedures for enhancing recruitment of reservoir fish stocks. The distribution and abundance of YOY fish populations in reservoirs are influenced by water levels, weather conditions, larval recruitment, interspecific competition, and other factors.

Temporal and spatial distribution of YOY shad (Dorosoma spp.) in reservoirs has been documented by many investigators (Houser and Dunn 1967, Netsch et al., 1971, Houser and Netsch 1971, Edwards et al., 1977, Craser 1979). However, the distribution of YOY of other fish species in reservoirs has received less attention. Krause and Van Den Avyle (1979) reported on the temporal and spatial distribution of larvae of principal fish species found in Center Hill Reservoir, Tennessee, and Kindschi et al. (1979) described the distribution of the larval fish species found in Rough River Lake, Kentucky.

Our purpose was to describe the population dynamics of YOY fishes in DeGray Lake during 4 years of epilimnial discharge, concentrating on the temporal and spatial distribution of shad, crappies (Pomoxis spp.) and sunfishes (Lepomis spp.).

STUDY AREA

DeGray Lake, located on the Caddo River in west central Arkansas, was impounded in 1969. At normal pool elevation of 124.4 m above sea level, the reservoir has an area of 5,427 ha, and maximum and mean depths of 57 m and 15 m. The reservoir extends in a west to northwest direction for about 32 km and has a shoreline length of 333 km.

Field data from DeGray Lake are being used to develop and test an ecosystem model developed by the Waterways Experiment Station, U. S. Army Corps of Engineers. For the assessment of spatial variation of selected biological and physical characteristics, the lake was divided into three sampling sections representing the upper, middle, and lower reaches (Figure 1). The upper section (1,238 ha) consists mainly of a narrow river channel bordered by small coves containing standing timber. The middle section (2,549 ha) includes open water areas and many large coves; two major tributaries flow into it. The lower section (1,661 ha) has large open water areas bordered by steep, rocky shorelines.

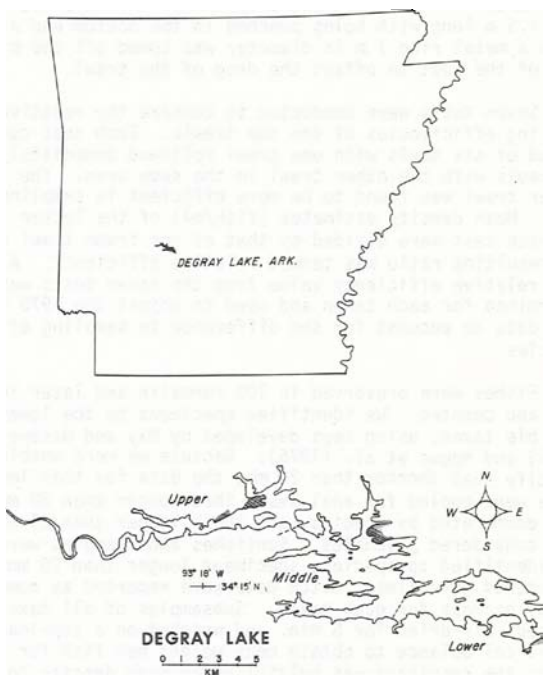


Fig. 1. Map of DeGray Lake, Arkansas, showing three sections of lake sampled.

METHODS

Although preliminary reservoir discharge samples were collected in 1975, regular sampling for larvae was not begun until early April 1976. We sampled YOY fish in the reservoir discharge weekly from the first week of April through July during 1976-78. Larval fishes were collected at a point 40 m downstream from the power house discharge with a townet, 3 m long and 1 m in diameter, with 0.79 mm (1/32 in.) mesh (Moen and Dewey 1976). During 1976, sampling periods were alternated weekly between morning (0930-1130 h) and afternoon (1300-1500). During 1977-78, samples were taken only in the afternoon (1300-1500).

Midwater trawling was used to determine temporal and spatial distribution of YOY fishes in the upper, middle, and lower sections of DeGray Lake. Young fish were sampled at 2-week intervals from May through July, 1975-78. Trawl-

ing sites were selected at random from a gridded map of the lake. Four hauls were made in the upper section of the lake, and eight hauls in each of the lower two sections. All sampling was done at night, because YOY fish have been shown to be more evenly distributed and nearer the surface at night than during the day (Netsch et al. 1971).

Trawling was conducted from an aluminum boat (8.5 m long, 3.2 m beam) powered by a diesel engine and fitted with two hydraulic winches. From May through June 1975 and 1976, a 1.88 m^2 frame trawl (Houser 1972) fitted with 0.79 mm mesh netting was used for sampling. When the mean length of shad approached 25 mm, a net with four panels having mesh sizes ranging from 12 to 4 mm (front to cod) was used on the same frame from the end of June through July 1975 and 1976. A sample consisted of an oblique haul from the surface to a depth of 7 m followed by retrieval. During the summer of 1977 and 1978, a 2 m^2 Tucker trawl with 0.50 mm mesh was used. It was lowered in a closed position to a depth of 7 m, opened, and retrieved at a 45° angle; an effective opening of 1.5 m^2 was maintained during retrieval. A General Oceanics flowmeter suspended in the mouth of both trawls was used to estimate the length of tows. The length of the tow was multiplied by the effective area of the opening of the net to determine volume of water sampled. Nets were retrieved at a speed of 0.9 m/s. The Tucker trawl was lowered off the starboard side of the boat, away from the boat and prop wash. A reinforced vinyl bag, 1.5 m long with holes punched in the bottom and attached to a metal ring 1 m in diameter was towed off the port side of the boat to offset the drag of the trawl.

Seven tests were conducted to compare the relative sampling efficiencies of the two trawls. Each test consisted of six hauls with one trawl followed immediately by six hauls with the other trawl in the same area. The Tucker trawl was found to be more efficient in sampling all taxa. Mean density estimates (fish/m^3) of the Tucker trawl for each test were divided by that of the frame trawl and the resulting ratio was termed relative efficiency. A mean relative efficiency value from the seven tests was determined for each taxon and used to adjust the 1975 and 1976 data to account for the difference in sampling efficiencies.

Fishes were preserved in 10% formalin and later identified and counted. We identified specimens to the lowest possible taxon, using keys developed by May and Gasaway (1975) and Hogue et al. (1976). Because we were unable to identify shad shorter than 20 mm, the data for that length range were pooled for analyses. Shad longer than 20 mm were enumerated by species, and those longer than 25 mm were considered juveniles. Sunfishes and crappies were not identified to species; specimens longer than 20 mm were considered juveniles. Catch data were reported as number/m^3 and as biomass for each taxon. Subsamples of all taxa were blotted, air-dried for 5 min. and weighed on a top-loading analytical balance to obtain mean weight per fish for each taxon; the resultant was multiplied by mean density to estimate biomass of each taxon. Total biomass (shad, crappies, and sunfishes combined) was calculated for each sampling data by section of the lake.

RESULTS

Twelve forms of YOY fish were collected: Gizzard shad, D. cepedianum; threadfin shad, D. petenense; crappies, sunfishes, brook silverside, Labidesthes sicculus; Mississippi silverside, Menidia audens; logperch, Percina caprodes; black basses, Micropterus spp.; minnows, Notropis spp.; flathead catfish, Pylodictis olivaris; catfishes, Ictalurus spp. and white bass, Morone chrysops. Shad, sunfishes and crappies made up more than 98% of the fish collected.

Temporal Distribution-- Larval shad were first collected in reservoir discharge samples during the second week of April in 1976 and 1978 and the third week of April in 1977. Catches of gizzard shad peaked earlier than those of threadfin shad, indicating that peak spawning of gizzard shad preceded that of threadfin shad. Gizzard shad have been reported to spawn at lower temperatures than threadfin shad (Kimsey 1958, Miller, 1960). Shad in the trawl samples were most abundant during the first week of June 1975, and during the last 2 weeks of May in 1976-78 (Fig. 2). A significant spawn of threadfin shad in late June or early July in 1976 resulted in large numbers of shad being present throughout the summer of that year.

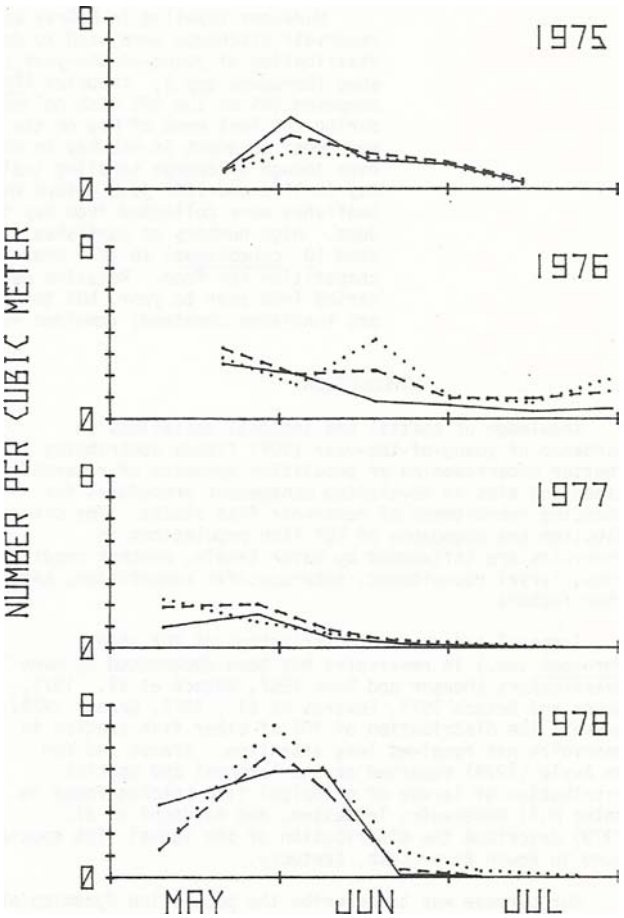


Fig. 2. Midwater trawl catches (No./m^3) of young-of-the-year shad from DeGray Lake in May, June, and July in 1975-78. Dotted lines represent the upper section of the lake, dashed lines the middle section, and solid lines the lower section.

Unusually cold winters during 1976-77 and 1977-78 with extended periods of water temperatures less than 7.2°C caused high mortality of adult threadfin shad, but had little apparent effect on adult gizzard shad. Threadfin shad have been found to be sensitive to low temperatures, with high mortalities usually occurring at 7.2°C (Parsons and Kimsey 1954). Few YOY threadfin shad were collected during either 1977 or 1978. Young gizzard shad were more abundant in May in both of these years than in 1975 or 1976. A mean shad density of 7.05/m^3 (composed almost entirely of gizzard shad) in the upper section on May 30, 1978 was the highest during the study. Nevertheless, total shad numbers was low by late June during both 1977 and 1978 because of the scarcity of threadfin shad.

Larval crappies appeared in reservoir discharge sampling during the second week of April of each year in 1976-78. Abundance in the discharge peaked in early May in 1976 and 1977, before midwater trawl sampling was started. In 1978, peak numbers of crappies occurred during mid May in both discharge and midwater trawl sampling. Densities of crappies collected by midwater trawling were considerably higher in 1977 and 1978 than in 1975 and 1976

(Fig. 3). This difference in abundance was apparent in the collection of juvenile crappies during summer cove sampling with rotenone in 1977 and 1978 (Multi-Outlet Reservoir Studies, unpublished data). Most crappies collected after the third week in June were juveniles.

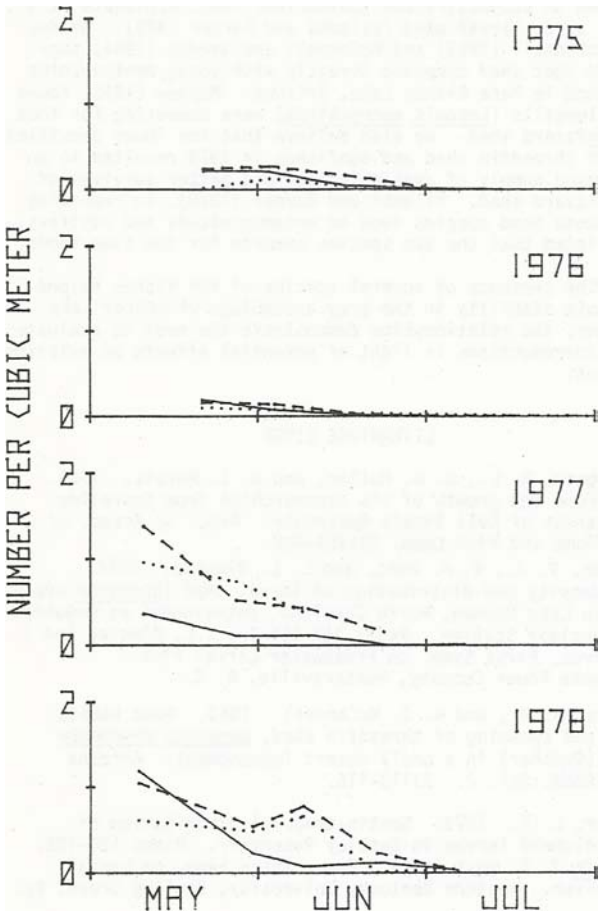


Fig. 3. Midwater trawl catches (No./m^3) of young-of-the-year crappies from DeGray Lake in May, June and July in 1975-78. Dotted lines represent the upper section of the lake, dashed lines the middle section, and solid lines the lower section.

Larval sunfishes first appeared in discharge collections during the first week in May 1976, and were usually most abundant in June each year. Sunfish densities were low and relatively similar in 1975 and 1976 (Fig. 4). In 1977, catches increased markedly from mid May through June and peaked again in July. A mean density of 10.27/m^3 in the upper section of the lake in June was the highest in 4 years of sampling.

Among the less abundant taxa, logperch were most numerous over the longest period of time (Fig. 5).

Spatial Distribution-- Larval shad were collected in all sections of the lake during May each year-- which suggests that spawning occurs lakewide. Mean densities of YOY shad were lowest in the upper section during 1975, and in the lower section during 1976. Threadfin shad densities were consistently higher in the upper area in 1975 and 1976. No consistent gradient in density by section of the lake was noted for shad during 1977 or 1978.

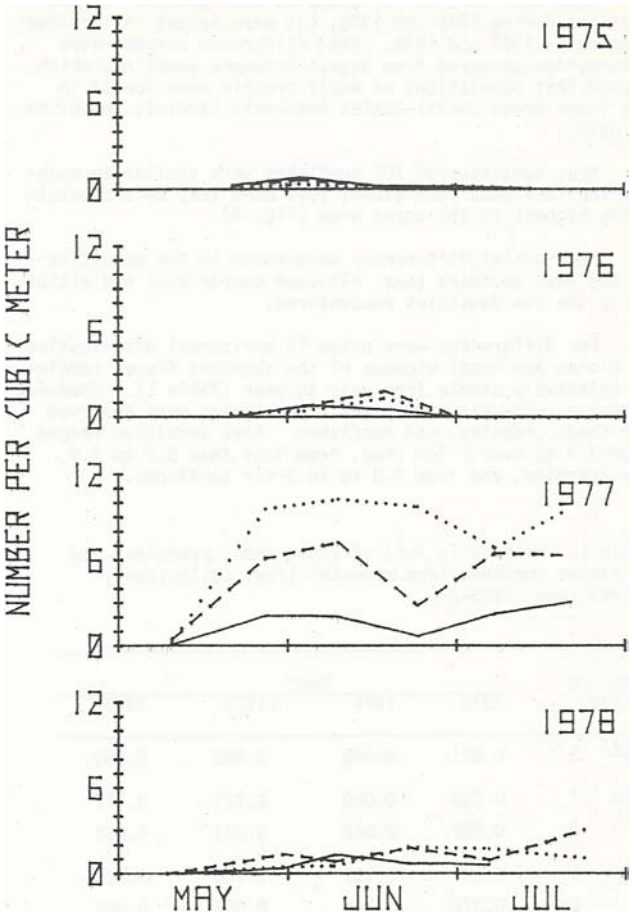
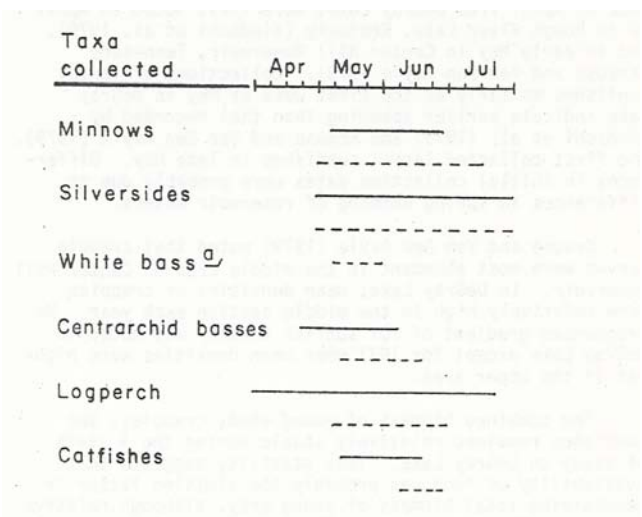


Fig. 4. Midwater trawl catches (No./m^3) of young-of-the-year sunfishes from DeGray Lake in May, June, and July in 1975-78. Dotted lines represent the upper section of the lake, dashed lines the middle section, and solid lines the lower.

Fig. 5. Seasonal occurrence of minor fish species (larval and juvenile) in DeGray Lake as indicated by reservoir discharge samples in 1976-78 (solid line) and midwater trawl samples in 1975-78 (dashed line).



a/ No white bass were collected in the reservoir discharge.

Mean densities of YOY crappies were similar for all sections during 1975 and 1976, but were lowest in the lower section in 1977 and 1978. That difference corroborated information gathered from August rotenone sampling, which showed that populations of adult crappie were lowest in the lower areas (Multi-Outlet Reservoir Studies, unpublished data).

Mean densities of YOY sunfishes were similar throughout the lake each year except 1977 when they were consistently highest in the upper area (Fig. 4).

Few spatial differences were noted in the densities of the less abundant taxa although comparisons are vitiated by the low densities encountered.

Few differences were noted in horizontal distribution of fishes and total biomass of the abundant fishes remained relatively stable from year to year (Table 1). However, large year-to-year variations in densities were observed for shad, crappies, and sunfishes. Peak densities ranged from 3.3 to over 7 for shad, from less than 0.2 to 1.4 for crappies, and from 0.8 to 10.3 for sunfishes.

Table 1. Biomass (g /m^3) of young shad, crappies, and sunfishes combined from midwater trawl collections, DeGray Lake, 1975-78.

Sampling Period		Year			
		1975	1976	1977	1978
May[a/]	1	0.021	0.048	0.088	0.090
June	1	0.068	0.060	0.121	0.117
	2	0.082	0.060	0.081	0.052
July	1	0.181	0.187	0.108	0.029
	2	0.076	0.194	0.053	0.007

a/ Comparable data for all four years was available for only one collection in May.

DISCUSSION

Initial spawning dates and seasonal abundance of YOY shad in DeGray Lake was similar to that reported by Netsch et al. (1971) for Beaver Lake, Arkansas during 1969 and 1970. Larval crappies, first collected during the second week of April from DeGray Lake, were first noted on April 30 in Rough River Lake, Kentucky (Kindschi et al. 1979), and in early May in Center Hill Reservoir, Tennessee (Krause and Van Den Avyle 1979). Collections of larval sunfishes as early as the first week of May in DeGray Lake indicate earlier spawning than that recorded by Kindschi et al. (1979) and Krause and Van Den Avyle (1979), who first collected larval sunfishes in late May. Differences in initial collection dates were probably due to differences in spring warming of reservoir waters.

Krause and Van Den Avyle (1979) noted that crappie larvae were most abundant in the middle area of Center Hill Reservoir. In DeGray Lake, mean densities of crappies were relatively high in the middle section each year. No pronounced gradient of YOY sunfish density was noted in DeGray Lake except for 1977 when mean densities were highest in the upper area.

The combined biomass of young shad, crappies, and sunfishes remained relatively stable during the 4 years of study on DeGray Lake. This stability suggests that availability of food was probably the limiting factor in maintaining total biomass of young prey, although relative densities of individual taxa fluctuated greatly as a result of variable reproductive success and early survival. For example, the decline in abundance of threadfin shad following the unusually cold winters of 1976-77 and 1977-78 was associated with marked increases in abundance of young sunfishes in 1977 and gizzard shad in 1978. Densities of gizzard shad were much lower in 1977 than in 1978. These annual differences in relative abundance of different taxa may have been partially influenced by competition for food resources, since larval and juvenile sunfishes are reported to feed on entomostracans (Sutherland 1953, Applegate et al. 1966) as do larval shad (Kilambi and Barger 1975). Gerdes and McConnell (1963) and McConnell and Gerdes (1964) suggested that shad competed directly with young centrarchids for food in Pena Blanca Lake, Arizona. Mayhew (1975) found YOY bluegills (Lepomis macrochirus) were competing for food with gizzard shad. We also believe that the lower densities of YOY threadfin shad and sunfishes in 1978 resulted in an increased supply of available food and better survival of YOY gizzard shad. Kilambi and Barger (1975), in reporting that both shad species feed on entomostracans and rotifers, postulated that the two species compete for the same foods.

The presence of several species of YOY fishes helped maintain stability in the prey assemblage of DeGray Lake. However, the relationships demonstrate the need to evaluate prey introductions in light of potential effects on existing species.

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FISH LARVAE CAUGHT BY A LIGHT-TRAP AT LITTORAL SITES IN LAC HENEY, QUEBEC, 1979 AND 1980

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ABSTRACT

Larval fishes were collected weekly during two summers (1979 and 1980) with a newly designed light-trap at two littoral sites in Lac Heney, Québec. Each sample was a 60-minute set of the light-trap in water 60-100 cm deep. Fifty percent of the 24 known species in the lake were captured at littoral sites as newly hatched and older larval stages. The samples were predominated by four or five species of Cyprinidae, two species of Percidae, one Centrarchidae, one Osmeridae and one Cyprinidontidae. Weekly samples demonstrated a succession of larvae resulting from periodic or protracted spawnings and hatchings in the lake; different species predominated at different times. Numbers of specimens in the 60-minute samples varied from six to 1135.

INTRODUCTION

The early life histories of most fishes found in Lac Heney have not been studied thoroughly, especially those spending some portion of their life among emergent and/or submerged aquatic plants. Scott and Crossman (1973) summarized the available information for the 24 species known living in Lac Heney and their results show need for further research on the early life of these fishes. In addition, the temporal occurrence of fish larvae can contribute considerable information about the annual phenology in Lac Heney.

Investigators normally utilize tow-nets to collect larval fishes (Amundrud et. al. 1974) but this study shows that light-traps can also be used. Tow-nets are effective in limnetic, oceanic and coastal marine regions but are difficult to fish in shallow littoral zones. Also, specimens collected with tow-nets are often mangled and difficult to identify. Although ecological studies of larval fishes living among aquatic plants are difficult, my success in capturing larval fish within a shallow bed of Scirpus suggests that light-traps can be useful tools in these studies. Moreover, the provision of live specimens by light-traps should increase opportunities for studies in physiology, behaviour, histology, etc. Although further research with light-traps is necessary to show the full extent of their worth, the results of this study demonstrated the usefulness of light-traps as collectors of live fish larvae.

METHODS AND MATERIALS

Free-swimming larval fishes were collected with a newly designed plexiglass light-trap at two littoral sites in Lac Heney, Quebec. The light-trap is described in detail in Faber (1981) but Fig. 1 shows a diagram of it. It consists of an upper light chamber, a lower animal chamber, a cylindrical section of netting, and a collection bucket. The light chamber holds the water-tight light bottle; the animal chamber has two vertical and two horizontal openings allowing animals to enter from four directions; the cylindrical section of netting joins the animal chamber to the collection bucket, which allows the operator to concentrate the catch into a small vessel. The bulb is powered by a 6-volt battery and its light intensity was measured in the lake at 2 lux at a distance of one meter (2300 hours, 18 July, 1980). The four animal entrances were set at a width of 3 mm and a length of 95 mm. Besides fish larvae, a number of other animal groups were collected including: Acarina, Amphipoda, Cladocera, Copepoda (Calanoida and Cyclopoida), Insecta (Coleoptera, Diptera, Ephemeroptera, Hemiptera and Trichoptera), Mysidacea, Oligochaeta and Ostracoda. The fish larvae needed to be hand-separated from these other animals.

Samples of larval fishes were collected weekly in 1979 and 1980 during June, July and August within the Lab Dock Study Area described by Faber (1980). The light-trap was allowed to fish for 60 minutes sometime between 2100 and 0030 hours. Thus, 60 minutes was the "unit-of-effort" for each sample. Samples were collected in 1979 at the end of a floating wooden dock in an opening of a shoreline bed of Scirpus validus Vahl. The depth of the water varied between 60 and 100 cm and the nearest leaf of S. validus Vahl. was 2 meters away. The growth of bullrushes was inhibited in this opening by boating activity. In 1980 the samples were taken within the bed of S. validus Vahl. approximately 10 meters to the east of the wooden dock. The trap was placed approximately one meter inside the outer edge of the Scirpus bed in water varying from 60 to 80 cm in depth. To avoid excessive disturbance at the sampling site, the trap was set and retrieved from the bow of a boat. Surface water temperatures were taken by a glass bucket thermometer.

I could identify many larvae from previous exposure to them in other studies but for others I needed to follow the development from larvae to juveniles. The observation of spawning activities in the lake and collection of some adults provided additional clues. The cyprinids caused most identification problems. Golden shiner larvae were identified by the presence of an unique ventral line of melanophores coupled with a dorsal series of paired melanophores and bluntnose minnow larvae were identified by their unique finfold morphology. The larvae of Notropis spp. could not be identified to species with certainty because two similar minnows are known to be present, i.e., N. heterodon and N. heterolepis.

Iowa darter, Etheostoma exile, larvae were identified by their typical percid morphology, unique pigmentation, and by studying a developmental series. Although several larval darters resemble this species, no other species of darter has been collected from Lac Heney. Yellow perch, Perca flavescens, and Iowa darter larvae are superficially similar at 6-8 mm but were separated by the following features: yellow perch possess air bladders, Iowa darters do not; yellow perch possess oblique lines of melanophores along myomeres in the posterio-lateral region, Iowa darters do not; Iowa darters possess a short vertical pigment line at the base of the pectoral fins, yellow perch do not.

All the specimens have been accessioned into the National Fish Collection of the National Museum of Natural Sciences (Accession number 1981-33).

Table 1. List of species and lengths of fish larvae taken from Lac Heney during the summers of 1979 and 1980 at two littoral sites with a light-trap.

Family	Species	$\bar{x}$ (mm)	Range (mm)	N	Year
Catostomidae:	White sucker, Catostomus commersoni (Lacépède)	13.6	13-15	40	1979
Centrarchidae:	Rock bass, Ambloplites rupestris (Rafinesque)	7.0	-	1	1980
	Pumpkinseed, Lepomis gibbosus (Linnaeus)	8.4	5-12	38	1979
	Pumpkinseed, Lepomis gibbosus (Linnaeus)	5.7	4-9	286	1980
Cyprinidae:	Golden shiner, Notemigonus crysoleucas (Mitchill)	5.0	4-6	2	1979
	Golden shiner, Notemigonus crysoleucas (Mitchill)	5.5	5-6	30	1980
	Bluntnose minnow, Pimephales notatus (Rafinesque)	6.6	5-12	72	1979
	Bluntnose minnow, Pimephales notatus (Rafinesque)	6.1	5-7	31	1980
	Minnows, Notropis spp [a]	5.6	4-12	183	1979
	Minnows, Notropis spp [a]	5.6	4-12	207	1980
Cyprinidontidae:	Banded killifish, Fundulus diaphanus (LeSueur)	6.6	5-12	184	1979
	Banded killifish, Fundulus diaphanus (LeSueur)	6.7	5-11	68	1980
Osmeridae:	Rainbow smelt, Osmerus mordax (Mitchill)	17.8[b]	13-25	1,291	1979
	Rainbow smelt, Osmerus mordax (Mitchill)	17.5[b]	15-25	978	1980
Percidae:	Iowa darter, Etheostoma exile (Girard)	7.0	3-13	446	1979
	Iowa darter, Etheostoma exile (Girard)	5.7	3-11	341	1980
	Yellow perch, Perca flavescens (Mitchill)	7.1	5-12	99	1979
	Yellow perch, Perca flavescens (Mitchill)	9.3	5-14	164	1980
	Walleye, Stizostedion vitreum (Mitchill)	11.0	-	1	1970

a. Two species may be present here, Notropis heterodon (Cope) and N. heterolepis Eigenmann and Eigenmann.
b. One hundred specimens from various dates were measured.

Table 2. Temporal succession of free swimming fish larvae at two littoral sites in Lac Heney, Quebec, during 1979 and 1980. Fish larvae were collected with a light-trap set for 60 minutes between 2100 and 0030 hours. Lines below numbers indicate presence of at least one larva with yolk material. Surface temperatures were taken with a glass thermometer at night.

Surface temperature (°C)		18	15	21	15	21	18	81	20	19	22	20	25	23	27	24	25	26	22	22	21	
Year of capture		79	80	79	80	79	79	80	80	79	80	79	79	80	79	80	79	80	79	80	79	TOTAL
Month of capture		Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jun	Jul	Jul	Jul	Jul	Jul	Jul	Jul	Aug	Aug	Aug	Aug	Aug	NUMBERS
Day of capture		3	6	10	14	17	22	22	30	6	8	10	17	18	24	27	2	3	17	12	23	
	(year)																					
Catostomus commersoni	(79)	40	-	0	-	0	0	-	-	0	-	0	0	-	0	-	0	-	0	-	0	40
	(80)	-	0	-	0	-	-	0	0	-	0	-	-	0	-	0	-	0	-	0	-	0
Stizostedion vitreum	(79)	0	-	1	-	0	0	-	-	0	-	0	0	-	0	-	0	-	0	-	0	1
	(80)	-	0	-	0	-	-	0	0	-	0	-	-	0	-	0	-	0	-	0	-	0
Perca flavescens	(79)	34	-	47	-	18	0	-	-	0	-	0	0	-	0	-	0	-	0	-	0	99
	(80)	-	78	-	83	-	-	2	1	-	0	-	-	0	-	0	-	0	-	0	-	164
Osmerus mordax	(79)	0	-	1	-	984	306	-	-	0	-	0	0	-	0	-	0	-	0	-	0	1291
	(80)	-	6	-	167	-	-	0	804	-	1	-	-	0	-	0	-	0	-	0	-	978
Etheostoma exile	(79)	3	-	181	-	23	23	-	-	10	-	6	8	-	142	-	17	-	20	-	13	446
	(80)	-	34	-	162	-	-	1	30	-	29	-	-	41	-	21	-	10	-	13	-	341
Notropis spp.	(79)	2	-	17	-	19	25	-	-	4	-	1	4	-	68	-	36	-	4	-	3	183
	(80)	-	0	-	55	-	-	1	41	-	37	-	-	47	-	3	-	16	-	7	-	207
Lepomis gibbosus	(79)	0	-	0	-	7	0	-	-	1	-	2	10	-	1	-	14	-	3	-	0	38
	(80)	-	0	-	0	-	-	0	43	-	6	-	-	148	-	24	-	39	-	26	-	286
Pimephales notatus	(79)	0	-	0	-	55	7	-	-	0	-	0	0	-	1	-	5	-	2	-	2	72
	(80)	-	0	-	0	-	-	2	16	-	3	-	-	9	-	1	-	0	-	0	-	31
Notemigonus crysoleucas	(79)	0	-	0	-	29	1	-	-	0	-	0	0	-	0	-	0	-	0	-	0	30
	(80)	-	0	-	0	-	-	0	0	-	0	-	-	2	-	0	-	0	-	0	-	2
Fundulus diaphanus	(79)	0	-	0	-	0	0	-	-	0	-	5	0	-	76	-	45	-	53	-	5	184
	(80)	-	0	-	0	-	-	0	0	-	5	-	-	3	-	1	-	41	-	18	-	68
Ambloplites rupestris	(79)	0	-	0	-	0	0	-	-	0	-	0	0	-	0	-	0	-	0	-	0	0
	(80)	-	0	-	0	-	-	0	0	-	0	-	-	0	-	0	-	1	-	0	-	1
Total Numbers		79	118	247	467	1135	362	6	935	15	81	14	22	250	288	50	117	107	82	64	23	4462

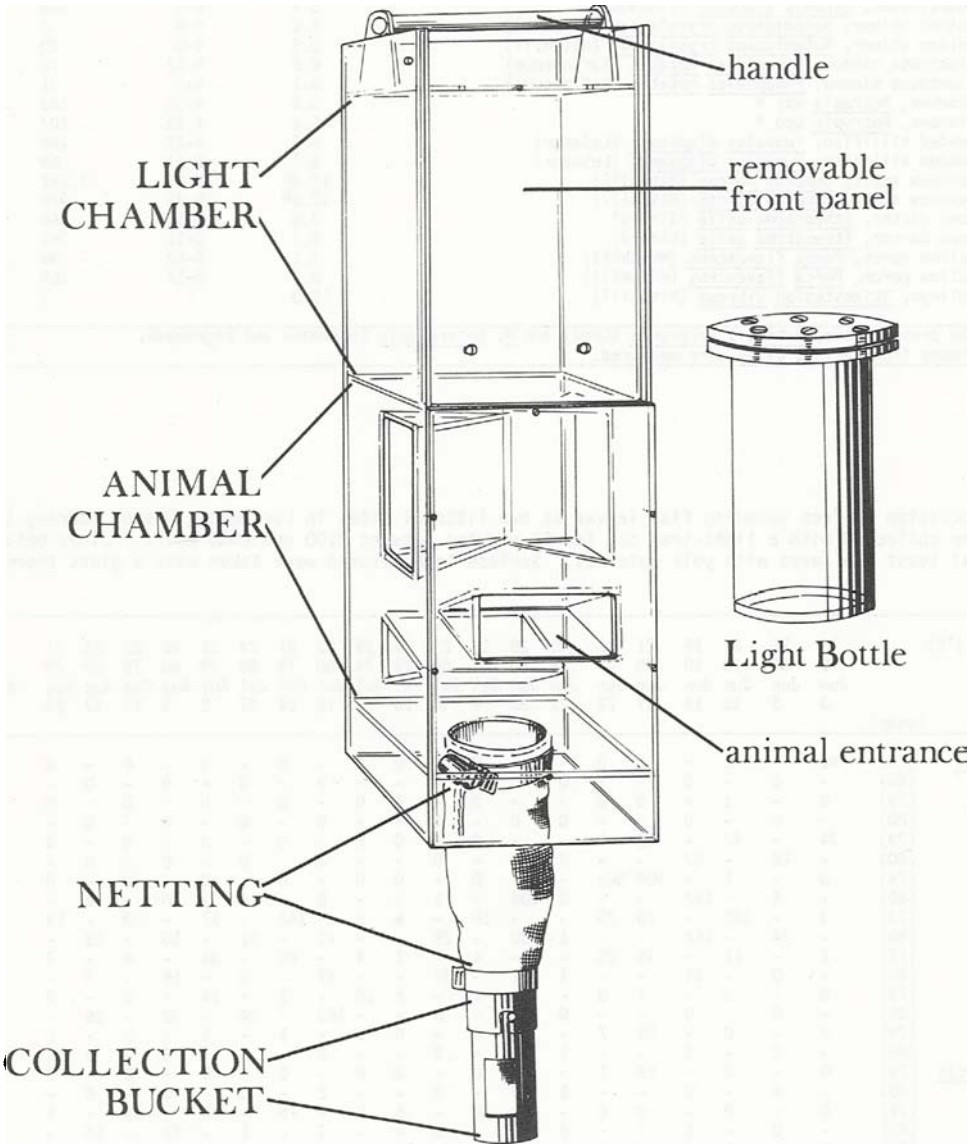

Fig. 1. Diagram of plexiglass light-trap. Light bottle is shown outside of light chamber without battery and bulb.

RESULTS

Species Composition: Numerous species were collected at the two littoral sites during the two-year period. Twelve or 13 species of fish larvae within 11 genera and 6 families were collected during the months of June, July and August (Table 1). Ten larvae were identifiable to species but the genus Notropis may have included more than a single species. Those 12 or 13 species represent 50% of the total number of fishes resident in the lake. Among them, six or more species spawn within rocky or weedy areas, three deposit their eggs in prepared nests and two lay their eggs in tributary creeks or freshets.

The range of sizes of larvae captured by the light-trap varied considerably (Table 1) and would probably have been greater if the trap's openings had been larger. Several juvenile cyprinids, rock bass and pumpkinseeds were seen near the trap and were observed to prey upon larval fish and other invertebrates swimming around the trap. The smallest larvae were Etheostoma exile which averaged 5.7 mm and 7.0 mm over the two years. In contrast, the largest larvae, Osmerus mordax, averaged 17.8 mm and 17.5 mm but ranged up to a maximum of 25.0 mm. Most larvae were captured by the trap shortly after hatching and many specimens possessed yolk material (Table 2) except Catostomus commersoni, Stizostedion vitreum, Osmerus mordax, Notemigonus crysoleucas and Ambloplites rupestris.

The number of specimens of each species varied considerably, ranging from one to 1291 in a single season. Some variation may be explained by the following: traps were set at two different sites; some species were more abundant than others in the lake; some species hatched before June and their patterns of abundance were missed, ie., white sucker and yellow walleye; while others remained in certain local and restricted sites, i.e., golden shiner. Illumination from the light-trap reached slightly outside the bed of Scirpus, so fish larvae from the deeper open water area were probably attracted and captured.

Two species not listed, (Coregonus artedii Le Sueur, lake herring, and Micropterus dolomieui Lacépède, smallmouth bass), were captured in other light-trap experiments. Other species known to occur in the lake but not taken by means of the light-trap include: Lake char, Salvelinus namaycush (Walbaum); lake whitefish, Coregonus clupeaformis (Mitchill); spoonhead sculpin, Cottus ricei (Nelson); deepwater sculpin, Myoxocephalus quadricornis (Linnaeus); trout perch, Percopsis omiscomaycus (Walbaum), northern pike, Esox lucius Linnaeus; brown bullhead, Ictalurus nebulosus (LeSueur); channel catfish, Ictalurus punctatus (Rafinesque); and ninespine stickleback, Pungitius pungitius (Linnaeus).

Temporal Occurrence: A continuum of spawnings and hatchings occurs each year in Lac Heney as a result of the presence of 25 different species of fish. This light-trap study in and near a bed of Scirpus from June through August monitored a portion of this continuum. The appearance and disappearance of fish larvae in the bullrushes produced a temporal succession of larvae during two summers in Lac Heney (Table 2). The patterns of temporal occurrences of fish larvae shown here were determined by a number of interacting biological and environmental factors, including: 1. species, 2. age and/or stage of development, 3. attraction to artificial lights, 4. nocturnal activity, 5. dispersal behavior, 6. presence in the area, 7. seasonal environmental changes at sampling site, 8. relative darkness of evening, 9. design of light-trap, and others.

Perch larvae appeared in increasing and then decreasing numbers during June in Lac Heney but they appeared during May, one month earlier, at similar surface temperatures in smaller lakes which warm-up faster in spring (Faber 1967; Amundrud et. al. 1974). Smelt larvae appeared and disappeared during June. Newly hatched smelt larvae were observed in limnetic regions during May and older larvae and juveniles were observed in June in immense numbers in certain sub-littoral regions (Faber, unpublished data). Common sucker and yellow walleye larvae were captured on only one occasion.

The second and third most abundant groups of larvae collected during the sampling interval were the Iowa darter and the minnows of the genus Notropis. Iowa darter larvae occurred throughout the period of sampling and their variable numbers did not delimit an obvious periodicity in spawning. Heretofore, darters were generally considered spring spawners (Winn 1958; Breder and Rosen 1966), but the occurrence of Iowa darter larvae with yolk from early June to early August suggests the spawning of Iowa darters in Lac Heney extends into summer. With an incubation period of 18 to 26 days (Bensley 1915), these data suggest that Iowa darters spawn continuously from mid-May through late-July, a full three months. The spawning period of the Iowa darter in Lac Heney appears unusually long compared to other known species of Etheostomatinae.

Larvae of Notropis spp, L. gibbosus and P. notatus appeared after the middle of June when surface water temperatures were 15°C. The occurrence of small and variable numbers of pumkinseed and bluntnose minnow larvae suggests continuous spawning in the area. Larvae of golden shiners were only collected on three occasions, yet they were continuously present in large numbers from late July through late August 10 to 20 meters away from the two sampling sites (Faber 1980). Killifish larvae were the last to make their appearance, during early July when surface water temperatures were 20°C. Only one rock bass larva was captured.

DISCUSSION

This study demonstrates that light-traps can be used to capture larval fish in shallow weedy areas. The number of species collected with light-traps is impressive but the total number of specimens is wanting. Fifty percent of the species known to be living in Lac Heney were represented in these light-trap samples. Gallagher and Conner (1980) reported they also collected about 50% of a total of 54 species in the lower Mississippi River with tow-nets, but suggested that certain larvae were more abundant in extrariverine areas (quiet vegetated areas) than in areas where they are able to tow. The larvae of yellow perch, smelt, Iowa darters, Notropis spp., pumpkinseeds, bluntnose minnows and killifish were dominant in the light-trap samples. Simultaneous tow netting in offshore limnetic areas of Lac Heney only captured yellow perch, smelt, Iowa darters and pumpkinseeds (Faber, unpublished data).

Tow netting in Lake Opinicon, (Amundrud et. al. 1974) resulted in the collection of large numbers of yellow perch (Perca flavescens), logperch (Percina caprodes), black crappies (Pomoxis nigromaculatus) and sunfishes (Lepomis spp.). The results from the studies in Lac Heney and Lake Opinicon suggest that a light-trap is more effective than a tow-net in capturing the larvae of Notropis spp., bluntnose minnows and killifish, but a detailed comparison between the collections of light-traps and tow-nets should be undertaken.

Various plexiglass traps designed to catch small fishes have been described in the literature. Some traps operate without lights and rely upon leads to catch fish (Breder Jr. 1960; Werner 1968; and Casselman and Harvey 1973), while others (Paulson and Espinosa Jr. 1975 and Kindschi et. al. 1979) including mine, rely upon lights to attract fish. Traps with and without lights were tested simultaneously on three occasions in Lac Heney and in each instance the trap with lights caught fish larvae while the trap without lights caught none. Paulson and Espinosa Jr. (1975) mentioned that they tested their trap during daylight hours and discovered that day sets were unsuccessful with or without lights. The main difference between their trap and mine, and the above described traps used without lights, is the presence of rectangular pieces of plexiglass used as leads. Apparently traps without lights and leads are ineffective as activity traps for larval or juvenile fish.

The success of light-traps depends upon fish larvae moving toward artificial lights. This behaviour is complex and not well understood. Some animals are attracted to artificial lights, some are unaffected, while others are repelled (Fraenkel and Gunn 1961). Moreover, the larvae and juveniles of certain fishes are attracted to artificial lights while adults are not (Verheijen 1958). Why do fish larvae or any other animals move toward artificial lights? Verheijen (1958) concluded that movements of animals towards artificial light sources are "forced movements", that is, they move toward the light because of photic disorientation rather than because of some inherent or learned behavioural activity.

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COMPARATIVE REPRODUCTIVE BIOLOGY OF THE THREADFIN AND GIZZARD SHAD IN LAKE TEXOMA, OKLAHOMA-TEXAS[1]

William L. Shelton,[2] Carl D. Riggs,[3] and Loren G. Hill

ABSTRACT

High reproductive potential and extended spawning period are desirable attributes for forage fishes but these qualities are seldom found in a single species. Threadfin shad (Dorosoma petenense) and gizzard shad (D. cepedianum) are two important forage species in southern waters; where they occur together they may not be totally non-competitive with each other. To define specific areas of overlap in their life histories, the reproductive ecology was assessed based on gonadal examination, spawning behavior, egg deposition patterns, and larval occurrence. In Lake Texoma, gizzard shad was found to initiate and terminate spawning earlier (March-May) than threadfin shad (April-June) but peak spawning for both species overlapped in early May. Water temperature of initial spawning for gizzard shad was 17 C compared to 19 C for threadfin shad. In contrast to gizzard shad, threadfin shad spawned nearer the water surface, spawning activity was more visible, and was concentrated in the early morning. Egg and larval identification was based on the eye-pigment characteristics of freshly hatched fry; threadfin shad have pigmented eyes at hatching while gizzard shad lack eye pigment until about 1 day after hatching.

INTRODUCTION

Fishery managers seldom find all desirable attributes of a forage fish in a single species. High fecundity is one such character; also, if spawning is protracted, it should result in a more continuous food supply for predators. Two closely related species may have complimentary characters which, if these species occur together, would increase and prolong the available food supply; however, habitat requirements may overlap in ways that could be detrimental to either or both species.

The threadfin shad (Dorosoma petenense) and the gizzard shad (D. cepedianum) are recognized as two important warmwater forage species. Each is prolific and if their spawning periods do not directly overlap, they may together be a more valuable food source than either species alone. Threadfin shad are predominantly schooling, limnetic, and planktivorous throughout life (Johnson 1970); gizzard shad are similarly characterized early in life but become more solitary, littoral, and are detritivores after their first year (Baker and Schmitz 1971). Competition between the species should be greatest during their limnetic, larval stages because they co-exist spatially and temporally (Netsch et al. 1971) and their food habits are similar (Cramer and Marzolf 1970; Van Den Avyle and Wilson 1980). This competition should be even greater if their spawning periods are simultaneous.

This study was conducted in Lake Texoma, a 41,710 hectare, U.S. Army Corps of Engineers hydro-electric/flood control reservoir on the Red River, Oklahoma-Texas. Primary effort was in the 400-hectare Buncombe Creek arm in Oklahoma. Physicochemical features were described by Sublette (1955).

The objective of the present study was to examine concurrently the reproductive biology of the two species in one water body to determine whether or not there were isolating mechanisms that minimized overlap. Gizzard shad have been in Lake Texoma since impoundment in 1942 (Riggs and Bonn 1959) but threadfin shad were not known to be present until about 1957 (Riggs and Moore 1958). At the time of this study, the latter had apparently replaced the former as the most abundant species in the reservoir but both species were thriving.

METHODS AND MATERIALS

In the present study, the reproductive biology was compared by ovary development, observations of spawning, and egg and larval sampling.

Minimum-maximum surface water temperatures were recorded daily near the mouth of Buncombe Creek arm at the University of Oklahoma Biological Station boathouse during 1968 and at weekly intervals during 1969; water temperature was also taken in conjunction with all other sampling.

Gonadal data are useful in determining the general spawning season and were used to compare these two colocated species. Ovarian development is of considerably greater value than testicular data because of more obvious seasonal changes. Gonadal-somatic indices (GSI = ovary weight/total body weight × 100) were calculated from fish of each species collected at weekly intervals with experimental gill nets and by electrofishing during the late winter and spring of 1968 and 1969. Ovaries from each species were weighed fresh to the nearest 0.1 gram--for gizzard shad longer than 215 mm (total length), based on initial maturity reported by Bodola (1966); for threadfin shad longer than 60 mm (Johnson 1971).

Spawning activity was observed on many occasions in conjunction with other sampling; however, spawning frequently occurred at certain locations and planned observations were made. Spawning of gizzard shad was most predictable and conveniently observed in the tributaries while threadfin shad spawning was easily studied at several Lake locations. Observations were usually taped on a portable recorder and later transcribed.

Devices designed to sample the adhesive spawned eggs (Shelton 1972) at natural spawning sites were placed in 12 designated areas of the Buncombe Creek arm during the 1968 and 1969 spawning seasons. Each sampler consisted of two 15 × 15 cm squares of welded iron rod fastened at a right angle to the other along an adjoining edge; each square frame was temporarily covered with fine-mesh (00) nylon webbing (225 cm^2) which was individually tagged. One sampling surface was thus oriented parallel to the substrate and the other was vertical. The procedure for using these samplers was as follows:

1. the Buncombe Creek arm was subdivided into 8 approximately equal sampling areas, two cove areas, and two in the flowing stream--one at the mouth and one about 2.4 km upstream from the mouth;
2. at a randomly picked site within each of the sampling areas, three samplers were set for periods of 48 hrs on alternate weeks;
3. one sampler was set on the bottom in water about 30 cm deep, one was set further offshore on the bottom in water 1.5 m deep (for bottom sets the horizontal square was parallel to the bottom and the vertical square perpendicular to it), and one was set floating with the top of the vertical square immediately beneath the surface of the water and the horizontal square parallel to the surface. This floating set was directly above the deeper bottom set;

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4. when the samplers were retrieved, the webbing surface was examined and those with adhering eggs were taken into the laboratory. Eggs on each surface were carefully removed, counted, and incubated for later identification. This practice was tedious and mortality prior to hatching was relatively high but we assumed a proportional loss for each species. The eggs of the two species cannot be distinguished from each other until hatching (Shelton 1978; Shelton and Stephens 1980).

In 1968, two floating samplers were also located at the Biological Station boathouse from 15 April until 5 June and checked daily.

Larvae were collected with a modified meter net (Gasaway and Lambou 1968); the webbing was 00 nylon. Surface hauls were made as close as possible to the shore; each sample was of 3-minute duration and transversed approximately 138 m. Trawling speed was not determined but was based on the approximate engine RPM. Trawling was done only in daylight and samples were taken on alternate weeks during the spring of 1968 and 1969 in each of the subareas of the study area as designated for egg sampling. From one to six samples were taken per subarea depending on the shoreline distance. Each sample was concentrated, preserved in 5% formalin, and examined in the laboratory. Shad larvae were separated from other species; identification of Dorosoma spp. was limited to yolk-sac larvae (Shelton and Grinstead 1973). The pigmentation of the eye of both species is developed after about 1 day and other differences do not develop until the larvae are 16-18 mm (Shelton 1972).

RESULTS AND DISCUSSION

Gonadal Development.--Ovaries of gizzard shad were examined from mid-February to mid-June in 1968 and 1969 (n = 198 and 203). The gonadal-somatic indices (GSI) increased slightly from mid-February through mid-March; by mid-March, in conjunction with increasing water temperature, yolk deposition had accelerated (Fig. 1). In early April of both years, ovarian development peaked with a modal GSI of between 7 and 10%. The mean surface water temperature during this period was 15-16 C. In both years, the mean GSI decreased slightly until early May and after mid-May of both years there was a significant decrease ($P < 0.05$) in GSI. The water temperature in early May of both years was between 18 and 21 C.

Ovaries of threadfin shad were examined from the same pre-spawning and spawning periods of each year (n = 239 and 206). Following a gradual increase through February and March, the GSI increased abruptly during the first two weeks of April (Fig. 1), while the mean temperature increased to approximately 15 C. Ovarian development peaked by mid-May (18-22%) when the water temperature was about 20-21 C. Gonadal-somatic indices were significantly reduced ($P < 0.05$) by late May when the water temperature was between 24 and 25 C. Ovaries of most threadfin shad were again near pre-spawning size by mid- to late June.

The ovaries of gizzard shad began to increase, peaked, and declined sooner within the year than that of threadfin shad. The GSI cycle for gizzard shad preceded that of threadfin shad by about one month. The peak GSI for gizzard shad was lower in both years than that for threadfin shad, but a direct comparison of reproductive potential of the two species is vitiated by the size difference of mature adults. Since the mature egg size for both species is the same (Shelton and Stephens 1980) and egg number per gram of body weight is similar (Kilambi and Baglin 1969; Johnson 1971; Jester and Jensen 1972), population fecundity could be compared only if population size and size distributions were known.

By late March and early April, ovulated eggs had collected in the ovarian lumen of gizzard shad and the GSI averaged 7-10% with an upper range of 16-18%; while threadfin shad did not have ripe eggs in the ovarian lumen until after mid-April and the average GSI in late April to early May was 16-20% with some as high as 26%. The release of mature eggs was reflected in a decline of GSI for gizzard shad in early May compared to a comparable decline in late May to early June for threadfin shad.

The seasonal pattern of the GSI suggests that

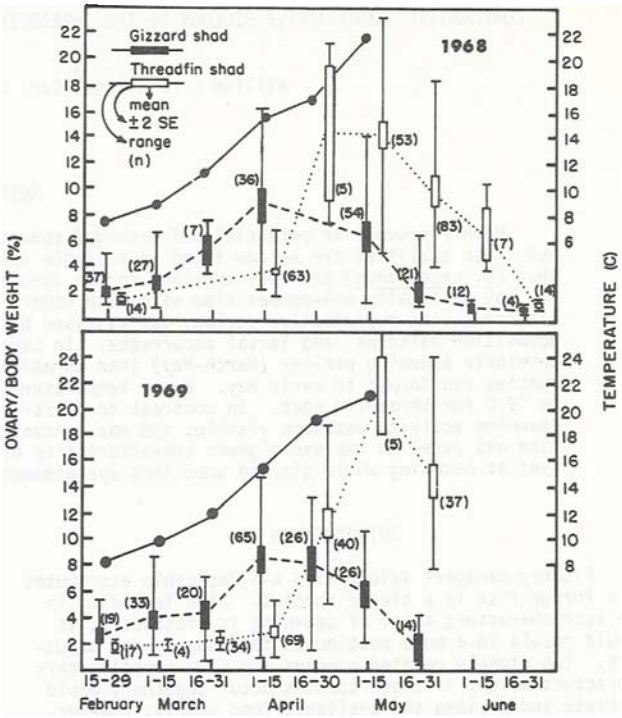

Fig. 1. Gonadal-somatic indices for gizzard and threadfin shad and mean daily water temperature (C) in 1968 and 1969 from Lake Texoma, Oklahoma-Texas. Dashed line = gizzard shad; dotted line = threadfin shad; solid line = temperature.

spawning for gizzard shad begins in late March to early April when the water temperature exceeds 15 C, reaching a peak in late April, and continuing until mid-May, for a total period of about 6-7 weeks. Baglin and Kilambi (1968) reported a comparable reproductive period for gizzard shad in Beaver Reservoir, Arkansas. Bodola (1966) reported about a 4-week spawning period for Western Lake Erie, when the temperature exceeded about 16 C.

Gonadal data for threadfin shad indicated an 8-10 week spawning period from mid-April to mid- or late June with a peak during the first part of May. The temperature at onset was about 19 C. Johnson (1969) found spawning of threadfin shad in Arizona reservoirs from April to June; based on gonadal data, Johnson (1971) reported that the older fish initiated spawning earlier in the season.

Observations of Spawning.--Many past descriptions of spawning were based on incomplete, chance observations. Our observations of threadfin shad spawning were planned on the basis of previous knowledge. However, gizzard shad spawning was predictable only in tributary locations.

Spawning by threadfin shad was first observed when surface water temperature reached about 19 C during the morning hours. During 1968, water temperature reached 19 C after noon on 3 or 4 days preceding the initial spawning. The first observed spawning was at 0900 on 18 April at a water temperature of 19 C. Activity continued for several hours, gradually diminishing until none was apparent after noon. We found no evidence for night spawning based on egg sampling and afternoon activity was only seen during initial spawnings of the season. During the latter part of April, spawning began about 1-2 hours after sunrise and was most intense between 0900 and 1200. By early May, spawning usually began within 15 to 30 minutes after sunrise and continued actively for only about 1-2 hours. This activity pattern was also evident from catches in surface-set gill nets and from eggs collected on floating samplers, both of which were suspended from the Biological Station boathouse. For example, on 4 May 1968, 1,890 spawning adults were netted between 0530 and 0700, 300 were captured between 0700 and 0830, and only 13 were collected in the remainder of the 24-hour period.

Eggs were collected from floating samplers in a pattern coincident with the abundance of adults. From 7-9 May, the water temperature did not warm past 19 C until well after sunrise and spawning was delayed about 1 hour compared to previous days.

Primary orientation of spawning groups of threadfin shad seemed to be toward the surface; during spawning, massive schools of threadfin shad were seen swimming parallel to the shore, periodically smaller groups would move shoreward. The smaller groups, composed of one to several females and many more males, were apparently led by females who selected the egg deposition sites. Upon encountering a suitable substrate, the group abruptly turned at the deposition site. Males crowded closer to the female and maneuvered erratically, which resulted in considerable surface agitation and eggs were scattered above and below the water surface on the substrate. The group continued swimming parallel to shore until another site was encountered or often they circled and repeatedly spawned on the same substrate.

Egg densities as high as 40-65/cm^2 were measured on egg-sampler surfaces. The most frequently used sites were in shallow littoral waters but pelagial floating objects were also frequently utilized.

The sex ratio in those spawning aggregations sampled was about 5 males to each female (4,495:879), and in general, the smallest ripe males were slightly smaller (≅ 60 mm TL) than ripe females (≅ 70 mm TL). Data on size distribution of spawning aggregations was discussed by Shelton (1972).

In contrast to threadfin shad, gizzard shad spawning was less easily observed. Occasionally, a group of gizzard shad would appear from deeper water, swim erratically shoreward along the bottom, abruptly turn, presumably having deposited eggs, and return to deeper water. Little surface disturbance was created during these spawnings.

Spawning in tributaries was more easily observed. Surface agitation was more common, perhaps because of the relative shallowness of the water. Spawning was probably initiated in the tributaries. Spawning occurred in daylight hours, but during periods of continuous observation, activity was more intense at night. During one 24-hour period on 21-22 April, spawning was most active from 2000 to 0500, gradually diminishing through 1200-1400, and was only intermittent for the remainder of the afternoon and early evening. Water temperature ranged from 16 to 21 C during this period.

Spawning aggregations in streams had from 40-50 adults of 20-36 cm TL (based on 159 fish collected on 28 March 1968). The sex ratio was 4:1 (males:females). The aggregation remained close-knit until a female moved upstream, or laterally, to deposit eggs; several males accompanied each female. The group then returned to the main aggregation. Concentrations of eggs on the substrate in tributary spawning appeared to be quite dense, although we did not sample those surfaces; however, the accumulation of gizzard shad eggs on samplers in the lake was rarely greater than 1 egg/cm^2.

On those occasions in which gizzard shad spawning was observed in the lake, it was similar to the behavior in the stream. However, it appeared that the large, mobile aggregations of spawning threadfin shad contrasted sharply with the smaller, less mobile groups of spawning gizzard shad.

The surface orientation of spawning threadfin shad permitted observation of the general movement along the shore but we cannot say whether or not a comparable activity occurs in gizzard shad. Judging from the spawning witnessed in the tributaries, a reasonable assumption is that gizzard shad aggregations were offshore in deeper water but shoreward movement along the bottom would not be obvious, particularly if the most active spawning occurred at night. The daylight spawning at the surface by gizzard shad reported by Jester and Jensen (1972) may not be typical of all populations. Bodola (1966) did not observe daylight spawning but had considerable evidence that most spawning occurred at night, with little surface disturbance. Thus, if this diel pattern is typical of gizzard shad, most lake spawning would be difficult to observe.

The spawning behavior of threadfin shad we witnessed agreed with those observations of Johnson (1971) but differed with aspects of other reports. Rawstron (1964) observed threadfin shad spawning at about 15 C while we observed a minimum temperature of 19 C. Hubbs and Bryan (1974) determined that the minimum incubation temperature for development of viable threadfin shad larvae was 15 C. Lambou (1965) described the abrupt cessation of threadfin shad spawning after sunrise while we observed a consistent diel pattern that was somewhat less restrictive.

Eggs and Larvae.--From 20 March to 12 July 1968, 236 egg samplers were examined, 14 of which were from the tributary area; from 10 March to 26 May 1969, 192 egg samplers were recovered, and 12 were from tributary sites. At the lake sites that had floating and bottom sets (deep and shallow), a total of over 66,000 Dorosoma spp. eggs was collected in 1968-69. Of those that hatched, 1,284 were gizzard shad and 2,269 threadfin shad (Table 1). A lower percentage of eggs taken from the shallow samplers hatched, but we assumed no differential mortality between species. Threadfin shad deposited the greatest number of eggs on the shallow bottom and floating samplers while gizzard shad deposited more eggs on the two bottom samplers (shallow and deep). Relatively few eggs were sampled from the tributary sites and none were identified as threadfin shad.

Table 1. Summary of shad eggs collected on lake samplers from March through July 1968 and 1969, Lake Texoma, Oklahoma-Texas.

Species	Eggs on samplers from each position						Totals
	Shallow		Floating		Bottom		
	(no.)	(%)	(no.)	(%)	(no.)	(%)	
Gizzard shad	808	63	138	11	338	26	1,284
Threadfin shad	1,863	82	375	17	31	1	2,269
Total collected[a]	61,483		2,917		2,331		66,731

[a]Only eggs that hatched could be identified.

Data from the floating sets at the boathouse (not included in Table 1) further suggest a difference in selection of egg deposition sites by the two species. A total of over 63,000 shad eggs was collected from the two samplers, 4,344 hatched and all were threadfin shad.

From 22 March to 29 August 1968, 323 meter-net samples were taken, 20 of which were from the tributary area; from 10 March to 14 June 1969, 109 samples were taken and 14 were from the tributary area. There were 2,099 larval gizzard shad and 448 threadfin shad yolk-sac larvae collected in the two years. The disproportionately high abundance of gizzard shad was a result of the tributary samples; 1,442 gizzard shad but no threadfin shad were collected in the creek.

The presence of yolk-sac larvae or eggs that later hatched was considered as indicative of the general spawning location and time of each species.

In general, eggs and larvae of gizzard shad were more commonly collected (89%) in the upper regions of the study area, which included the main tributary that entered that portion of Buncombe Creek arm (Table 2). In the segment of the stream that was sampled, no threadfin shad egg or larva was collected, while the highest density (52%) of the gizzard shad eggs and larvae came from this limited area. In contrast, a greater proportion of threadfin shad eggs and larvae (93%) was collected in the lower half of the arm. This region was adjacent to the open expanse of the main reservoir, a habitat apparently more frequented by identifiable larval threadfin shad.

The initiation of spawning by most temperate fishes is closely correlated with water temperature and initial spawning is usually observed in the suitable habitat that warms earliest. No larvae or eggs were collected in the lake prior to 1 April of either year (Table 3) but in 1968 gizzard shad spawning was first observed in the tributary on 27 March at a water temperature of 17 C while the lake temperature was 13 C.

Table 2. Combined numbers of eggs and yolk-sac larvae of gizzard shad and threadfin shad collected by regions during 1968 and 1969.

Region	Gizzard shad		Threadfin shad	
	(no.)	(%)	(no.)	(%)
Buncombe Creek[a]	1,796	52	0	0
Upper Section[b]	1,267	37	182	7
Lower Section	384	11	2,545	93

[a]Sample site 2.4 km upstream in tributary.
[b]Upper one-half of Buncombe Creek arm.

Table 3. Combined numbers of eggs and yolk-sac larvae of gizzard and threadfin shad collected by time periods during 1968 and 1969, Lake Texoma, Oklahoma-Texas.

Species--Year	March		April		May		June	
	1-15	16-30	1-15	16-30	1-15	16-31	1-15	16-30
Gizzard shad								
1968	-	5	581	1,775	438	2	0	0
1969	0	0	270	20	330	26	0	-
Threadfin shad								
1968	-	0	0	28	395	36	510	47
1969	0	0	0	25	109	1,561	16	-

During the period 1-15 April, eggs but no yolk-sac larvae of gizzard shad were collected from the lake but larvae were abundant in the tributary; neither eggs nor larvae of threadfin shad were collected in this period. From 16-30 April, eggs and larvae of both species were sampled in the lake with those of gizzard shad predominating. In 1968, threadfin shad spawning was first observed on 18 April when the water temperature was 19 C; eggs were collected at that time but larvae were not taken until 24 April.

During the first half of May, most yolk-sac larvae collected were threadfin shad, although gizzard shad were still spawning as their eggs were quite abundant in the samples. During the last half of May, trawling was hampered because precipitation had carried debris into the lake. The resulting high water also complicated placement and recovery of egg samplers; however, threadfin shad larvae and eggs predominated. From 1-15 June, only threadfin shad were collected; by late June their abundance had declined and after 1 July neither eggs nor larvae of either species were sampled.

Gizzard shad apparently initiated spawning earliest in the inflowing stream where the water temperature warmed sooner. Gizzard shad spawned from late March, beginning in the tributary, through late May or about 7-9 weeks. Threadfin shad did not ascend the tributary but began spawning in the lake in mid- to late April and continued through late June, for an estimated duration of 8-10 weeks. Those spawning periods are slightly longer than those estimated from gonadal data and clearly illustrate temporal overlap with slightly different peaks. A temporal spawning difference of about one month was also described for the two shad species in Beaver Reservoir based on larval fish sampling (Netsch et al. 1971; Houser and Netsch 1971).

Threadfin shad spawning was more concentrated near the open water area while gizzard shad reproduction was more common in the upper section of the arm. Netsch et al. (1971) found gizzard shad larvae several weeks earlier than threadfin shad in the upper portion of Beaver Reservoir, Arkansas.

A further spatial separation in spawning sites was indicated by the egg deposition patterns. Both species deposited eggs in the shallow water but threadfin shad eggs were more frequently found on the floating samplers, suggesting a surface orientation, whereas gizzard shad eggs were proportionately more abundant on the deeper-bottom samplers. Jester and Jensen (1972) reported surface spawning activity by gizzard shad (around a boathouse) similar to that described for threadfin shad in our study, but we did not see comparable activity. The floating egg samplers used in our study at the boathouse not only provided a convenient daily check of spawning for threadfin shad but confirmed that none of the eggs collected there were those of gizzard shad.

SUMMARY AND CONCLUSIONS

Interspecific competition cannot be proved by simply demonstrating overlapping use of a common resource. Conversely, detailed study of mechanisms that segregate aspects of the life histories of two closely related species may suggest that an apparently limited resource is used by each species differently in time or space and is indeed not limiting. Gizzard and threadfin shad appear to be similar biologically, especially in their early life histories. Based on gonadal development, observations of spawning, and egg and larval collections in Lake Texoma, we concluded that there was overlap in seasonal, diel, and spatial aspects of reproduction, but we observed probable isolating (segregating) mechanisms of reproduction in the two species, as well (Table 4).

Table 4. Summary of differences in the reproductive biology of threadfin and gizzard shad in Lake Texoma, Oklahoma-Texas.

Characteristic	Gizzard	Threadfin
GSI		
Peak (%)	7-10	16-20
Time	early April	early May
Spawning		
Initial	late March--16 C	late April--19 C
Duration (wks)	7-9	8-10
Size (mm TL)		
male	200-?	60-170
female	215-360	80-190
Sex ratio (♂:♀)[a]	2-4:1	5-15:1
Orientation	bottom	surface
Diel peak	nocturnal	early morning
Eggs (mm)[b]	0.9-1.1, adhesive	0.9-1.1, adhesive
Incubation (hrs--23 C)[c]	43	72
Larval eye[c]	non-pigmented at hatching	pigmented at hatching

[a]Shelton (1972).
[b]Shelton (1978).
[c]Shelton and Stephens (1980).

The duration of peak spawning for gizzard shad was April through early May, whereas for threadfin shad it was principally during May. Gizzard shad spawned earlier in the season when surface waters were about 16 C compared to about 19 C for the threadfin shad. Gizzard shad spawned earliest in the tributary compared to the lake proper. That threadfin shad did not spawn in the tributary may have been related to the small size of the stream and spawning by this species in larger lotic systems elsewhere may not be limited.

Even during the overlapping peak spawning period, however, there appeared to be more spawning by threadfin shad near the open water portion of the arm. Even though both spawned in regions of shallow water, separation was somewhat effected by the preference for bottom substrates by gizzard shad and for emergent substrates by the threadfin shad. Moreover, threadfin shad showed a very definite early morning activity while gizzard shad spawning activity was greater during hours of darkness. Differences in diel activity would tend to reduce the overlap and thus

hybridization, even though natural hybrids are produced (Shelton and Grinstead 1973).

Probably the most important consideration with reference to potential competition by larvae of the two species was the seasonal difference in spawning peaks. To the fishery manager, the presence of small prey over a longer period of time than would be the case if only one species were present is probably one of the more significant aspects of their relative reproductive biology and therefore it is possibly advantageous to have both of these species as prey in a system.

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SURVIVAL OF SEVERAL SPECIES OF FISH LARVAE AFTER PASSAGE THROUGH THE LUDINGTON PUMPED STORAGE POWER PLANT ON LAKE MICHIGAN

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Survival of fish larvae was studied from 1979-1980 in association with entrainment and passage through the Ludington Pumped Storage Power Plant (LPSPP) on Lake Michigan. A new sampling technique consisting of slow vertical hauls with a 2 m-diameter plankton net was evaluated and found to effectively capture live fish larvae. A total of 171 samples was collected from two control and two power plant discharge stations. Alewife and smelt dominated the 2138 larvae collected. Other taxa included: yellow perch, ninespine stickleback, johnny darter, burbot, lake whitefish, bloater, deepwater sculpin, Cyprinidae, and Cottus. Alewife exhibited low initial survival rates (17% control and 11% experimental), but improved methods indicated survival may be as high as 40-44% at experimental sites. Small (≤ 8 mm) larval smelt exhibited 57% and 50% initial survival at the control and power plant sites, respectively. Higher initial survival was recorded for large smelt larvae (≥ 8 mm) at the control sites (91%), compared to 43% at the discharge sites. Latent mortality further decreased large smelt survival to 11% at the discharge sites. Most other species exhibited high survival (80-100%) at the control and discharge stations, though estimates were derived from fewer specimens especially in the discharge samples. Initial survival for all species combined was 67.5% at control stations compared to 38.7% at discharge stations Survival of larvae 24 hours after capture was 71.9% for the control site and 53.0% for the discharge site.

Recent power plant studies (Boreman 1977; Goodyear 1977) suggest greatest impacts to fish communities may result from the entrainment of nonscreenable organisms (e.g fish eggs and larvae). However, few studies examining power plant related mortality exist. Marcy (1971, 1973) found larval mortality at nuclear plants primarily due to mechanical damage or prolonged exposure to heated effluent. Historically, 100% mortality has been assumed at fossil fuel plants, but Ecological Analysts (1976) observed 25-50% survival for clupeid and percichthyid larvae, though survival of larval atherinids was essentially zero. Studies at hydroelectric pumped-storage plants by Snyder (1975) and Prince and Mengal (1980) have indicated survival of entrained fish larvae may be substantial Laboratory studies conducted by Beck et al. (1975) indicate high survival rates for striped bass eggs and larvae subjected to pressure regiments that simulated passage through a pumped storage plant.

One of the most critical factors affecting survivorship studies is sampling mortality. Most mortality associated with collection can be attributed to mechanical damage, especially impingement and abrasion, against plankton nets deployed in strong currents. Recent technological developments (McGroddy and Wyman 1977) have introduced the larval fish table which is effective at reducing water velocities and thus increasing larval survival during collection but is cumbersome, relatively immobile, unable to draw water up a large head and unable to adequately sample larvae present in low densities because of small volume of water delivered.

Cada and Hergenrader (1978) developed regression equations to discriminate between entrainment mortality and net-induced sampling mortality. They found a direct relationship between observed mortality and water velocity in the nets at control stations. Further, they recognized the importance of maintaining similar physical conditions (especially current velocity) in intake and discharge samples.

Gear deployed in power plant research must often be site selective and the purpose of this study was to develop a technique that could rapidly sample sufficient numbers of larvae at several sites to directly evaluate survival. A slow vertical haul with a large diameter (2 meter) plankton net was used to reduce current across the net and filter enough water to collect sufficient numbers of larvae for study. Research was conducted at the Ludington Pumped Storage Power Plant on Lake Michigan.

DESCRIPTION OF STUDY AREA AND POWER PLANT

The Ludington Pumped Storage Power Plant (LPSPP) is located on the east-central shore of Lake Michigan 6.5 km south of Ludington, Michigan. The six intakes of the LPSPP are located on the dredged out shoreline of Lake Michigan and extend from 11.6 m below the surface to 21.4 m at bottom (Figure 1). Screening consists of a gridwork with 30.5 x 58.4 cm openings to prevent entry of large debris. Each intake is attached to a Francis-type reversible turbine which transfers water between Lake Michigan and the upper reservoir (150 m above Lake Michigan). Water passes through 8 m diameter penstocks that are 396 m long and open at a depth of 23 m and extend to the bottom of the upper reservoir. Maximum water velocities in the penstocks approach 8.5 m/sec, which indicates larvae are present in the power plant system for a minimum of 47 seconds in transfer between the two bodies of water. During this time they are exposed to rapid pressure changes of 1-11 atmospheres.

The upper reservoir is 30 to 34 m deep and encompasses 332 hectares at full pond and fluctuates daily up to 20.4 m during routine generation. Sides are asphalt lined down to 23 m, with 3 m of compacted clay on the bottom. During generation, water empties into Lake Michigan between two rock jetties 335 m apart composed of large limestone boulders which extend 490 m from shore. Water velocities 122 m out from the intake (where entrainment and mortality samples were taken) average 50-100 cm/sec, and are reduced to 20-30 cm/sec at the end of the jetties.

METHODS

Survivorship sampling was conducted approximately every two weeks from late April through August in 1979 and 1980 in the upper reservoir discharge during pumping mode (night), in the Lake Michigan discharge between the jetties during generation mode (day), and at a control site approximately 1.6 km south of the plant (day and

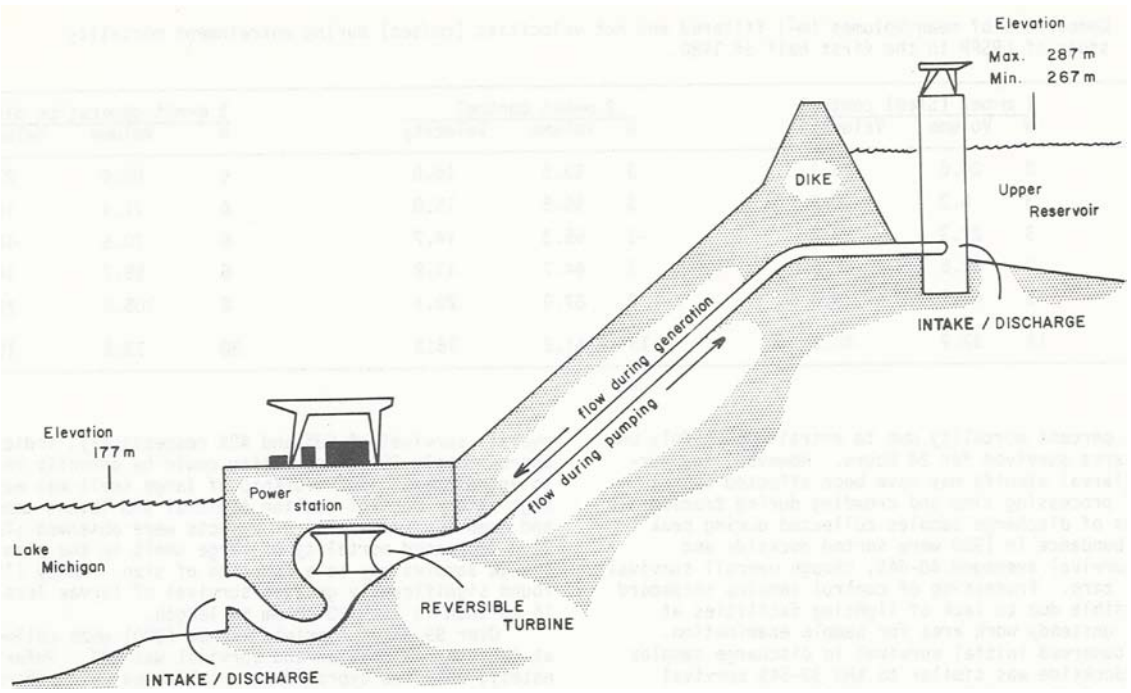

Figure 1. Schematic illustration of the Ludington Pumped Storage Power Plant showing elevations, intake-discharge locations and flow directions.

night) (Figure 2). Sampling gear consisted of a 2 m-diameter 351μ mesh conical plankton net with a mouth to length ratio of 6:1, equipped with a General Oceanics flow-meter (Model 2030) mounted one-third off center in the mouth. Initially all samples were collected by making a slow bottom to surface vertical haul using a Mytewinch connected to a 12 volt battery. Difficulties in maintaining a vertical haul in the plant discharge were successfully overcome by utilizing boat power and the swift currents emanating from the plant to position the boat directly over the net. Control samples were taken identically at the 9.1 m contour to approximate the depth of plant discharge samples. However, larvae of certain taxa were scarce at this depth (stratum) in 1979 which prompted additional control sampling in 1980 using a 1 m-diameter, 351μ mesh plankton net mounted on a sled and towed at the 1.6 m depth contour for two minutes.

All samples were immediately returned in 19 liter plastic buckets to the laboratory and sorted in enamel trays under lighted magnifying lenses. Live fish were placed in 11.5 liter aquaria and maintained at ambient collection temperature in water baths provided by a Living Stream (Model LS-700). Dead fish were preserved immediately in Davidson's solution (Lam and Roff 1977). Locomotor activity was the criterion for live/dead determination. Initial survival refers to larvae alive at time of sorting, latent survival refers to larvae surviving 24 hours in aquaria, and overall survival is the number of larvae alive after 24 hours divided by the total number of larval collected. Power plant samples were usually processed within one hour of collection, however control samples required more time due to greater distance traveled. During peak alewife concentrations in 1980, plant discharge samples were sorted dockside and usually completed within 15 minutes of collection.

Control samples were analyzed statistically with the Mann-Whitney U test (Sokal and Rohlf 1969) to determine if survival of larvae differed significantly between years. Tests on all taxa indicated survival was similar ($\alpha = 0.1$) and data were pooled from 1979 and 1980.

RESULTS AND DISCUSSION

Data collected in the first half of 1980 indicated that the volume of water sampled and current velocity were similar between the control and experimental samples taken with the large diameter plankton net on a slow vertical haul (Table 1). Further, mean current velocities within the net (15-19 cm/sec) during sampling were markedly less than currents in the discharge area which were greater than 50 cm/sec nearly 75% of the time (Liston et al. 1980). Samples collected with the one meter net attached to a sled strained somewhat smaller volumes of water, but sampling velocities were greater due to the necessity of horizontal towing by boat. However, sled samples were necessary to evaluate survival of certain species of larvae (lake whitefish, burbot, cyprinids) which were virtually absent in samples taken at deeper contours (Liston et al. 1980). Data analysis indicated larval survival was not affected by the increased velocities of the sled towed net.

During the two year study, a total of 171 samples was collected at the discharge (89) and control (82) sites. A total of 2138 larvae represented at least eleven species and seven families (Table 2). Most larvae (86.2%) were taken at the control site, and greater survivorship (all species combined) was observed there, though survival at both sites was substantial. However, survival was species specific and, for at least one species, size related. These phenomena were also reported by Cannon et al. (1978). Analysis of survival by species is given below. Statistical comparisons were not made because of low numbers collected of most taxa.

Alewife (Alosa pseudoharengus) comprised 33% of all larvae collected (Table 2). This species exhibited lowest initial survival of any larvae both in the control (17.3%) and experimental (11.8%) samples suggesting at

Table 1. Comparison of mean volumes (m^3) filtered and net velocities (cm/sec) during entrainment mortality study of LPSPP in the first half of 1980.

Date	1 m-net (Sled) control			2 m-net control			2 m-net generation discharge		
	N	Volume	Velocity	N	Volume	Velocity	N	Volume	Velocity
4-30-80	2	24.6	26.1	3	63.5	16.8	6	82.0	21.8
5-12-80	3	8.3	8.8	3	56.5	15.0	6	71.4	18.9
5-27-80	3	27.3	29.0	3	55.3	14.7	6	70.6	18.7
6-10-80	2	82.6	87.6	3	44.7	11.8	6	55.7	14.8
6-26-80	3	47.1	50.0	3	87.0	23.1	6	105.6	28.0
TOTAL	13	37.9	40.2	15	61.2	16.2	30	73.2	19.4

least 5.5 percent mortality due to entrainment. Only one alewife larva survived for 24 hours. However, low survival of larval alewife may have been affected by length of processing time and crowding during transport. Two series of discharge samples collected during peak alewife abundance in 1980 were sorted dockside and initial survival averaged 40-44%, though overall survival was still zero. Processing of control samples shipboard was impossible due to lack of lighting facilities at night and unsteady work area for sample examination. However, observed initial survival in discharge samples examined dockside was similar to the 33-54% survival reported by Ecological Analysts (1976) and Cannon et al. (1978) in the discharge of fossil fuel and nuclear plants, and by Prince and Mengel (1980) at a pumped storage site in South Carolina for clupeids.

Smelt (Osmerus mordax) was the most abundant taxon collected in survival samples and comprised 40% of the collections. Preliminary data analysis indicated survival for larvae and juveniles larger than 8 mm was greater at the control site. Small smelt showed similar survival rates at the control and discharge sites (58% and 50% respectively; Table 2). Latent survival was high and similar between control and discharge sites for an overall survival of 53% and 42% respectively, indicating approximately 10% of mortality could be directly related to entrainment. Survivorship of large smelt was extremely high at the control station (initial and latent samples), and even less after latent effects were observed (Table 2). Increased mortality of large smelt in the plant discharge samples may be a function of size. Marcy (1973) found significantly greater survival of larvae less than 15 mm than in fish 20-40 mm in length.

Over 99.3% of cyprinid larvae (300) were collected at the control station and survival was 88%. Unfortunately, only two cyprinid were collected in discharge samples and one survived the 24-hour observation.

Larval yellow perch (Perca flavescens) were collected in low numbers at all sites (Table 2). Survival rates were 33% at the control site and 14% at the discharge site, indicating 19% entrainment mortality. All live larvae survived 24 hours.

Most other larval taxa (burbot, Lota lota; sculpins, Cottus sp.; johnny darter, Etheostoma nigrum; lake whitefish, Coregonus clupeaformis; ninespine stickleback, Pungitius pungitius: and bloater, Coregonus hoyi) had high (80-100%) initial survival in both control and

Table 2. Survival of fish larvae (of several species) entrained at the Ludington Pumped Storage Power Plant and collected at a control station on Lake Michigan 1979-1980.

Taxa	Initial Survival				Latent Survival			
	Control		Discharge		Control		Discharge	
	N	Percent (*) Alive	N	Percent (*) Alive	N	Percent (*) Alive	N	Percent (*) Alive
Alewife	567	17.3(9.9)	132	11.8(16.8)	98	1.0(11.8)	18	0(0)
Smelt (≤ 8 mm)	38	57.9(32.2)	60	50.0(32.2)	20	90.0(94.6)	6	83.3(58.8)
Smelt (> 8 mm)	712	91.6(12.2)	37	43.2(41.3)	42	85.7(46.3)	16	31.2(33.7)
Fourhorn sculpin	3	67.7(157.8)	14	78.6(24.2)	2	0(0)	11	9.1(19.5)
Burbot	5	100.0(0)	6	100.0(0)	5	100.0(0)	6	100.0(0)
Cottus sp.	0	-	16	81.2(38.8)	0	-	13	92.3(8.4)
Johnny darter	111	90.1(7.1)	12	100.0(0)	100	97.0(5.9)	12	83.3(26.3)
Yellow perch	9	33.3(36.0)	7	14.2(19.4)	3	100.0(0)	1	100.0(0)
Lake whitefish	33	100.0(0)	3	100.0(0)	33	100.0	3	100.0(0)
Ninespine stickleback	63	100.0(0)	7	85.7(29.4)	63	100.0(0)	6	100.0(0)
Cyprinidae**	300	88.0(18.3)	2	50.0(71.3)	264	86.0	1	100.0(0)
Bloater	1	100.0(0)	0	-	1	100.0(0)	0	-
TOTAL	1842	67.5%	296	38.7%	631	71.9%	93	53.7%

*90% Confidence Interval
**Primarily spottail shiner

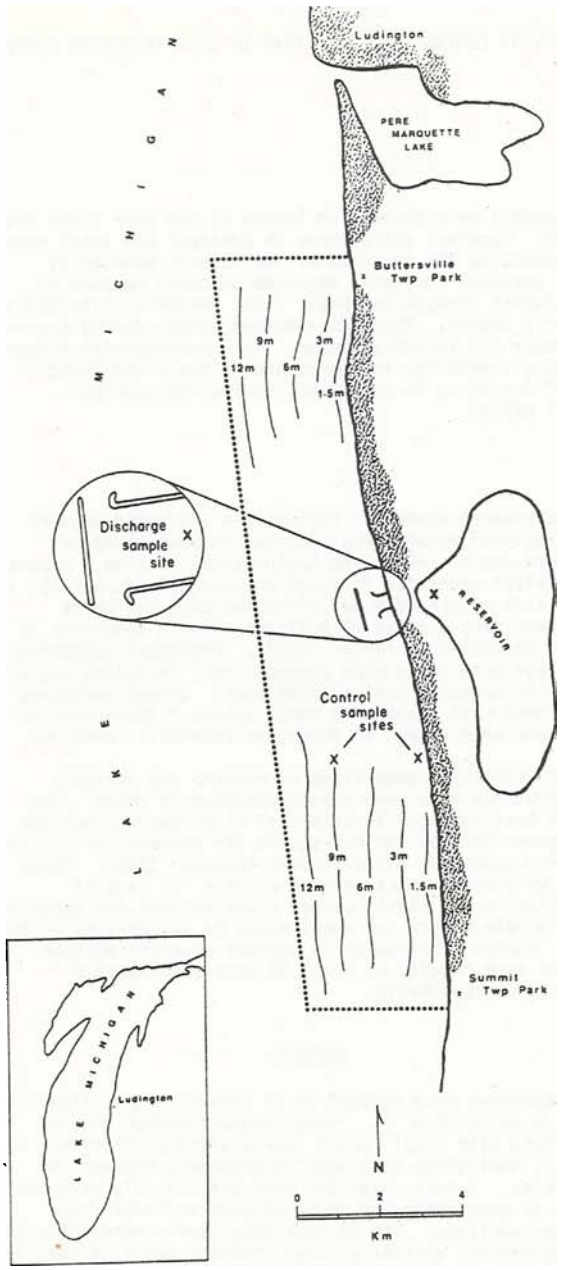

Figure 2. Schematic diagram illustrating control and discharge sample sites.

discharge samples, but few larvae were collected in discharge samples. Survivorship was 100 percent after 24 hours for burbot, lake whitefish, ninespine stickleback, and bloater larvae. Initial survival of johnny darter larvae was slightly higher in discharge samples (100%) than in control samples (90.1%), but observation after 24 hours revealed a 14 percent lower survival in discharge samples (Table 2). The overall survival of 87% in control samples and 83% in discharge samples, indicated 4% entrainment mortality.

Initial survival of fourhorn sculpin (Myxocephalus quadricornis) was greater in discharge than in control samples (Table 2). Latent survival was low as only a single larva survived the 24-hour holding period. These data suggest that fourhorn sculpin larvae are relatively intolerant to handling.

In conclusion, use of a large (2 m) diameter plankton net retrieved vertically appears to be an effective method for collecting live larvae of several species of fish. More fragile larvae (e.g. alewife) must be removed from samples quickly to ensure accurate live/dead determination. This sample technique has the advantages of mobility, reduction of net velocity, and ability to sample large volumes of water.

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CORRESPONDENCE OF MYOMERES AND VERTEBRAE AND THEIR NATURAL VARIABILITY DURING THE FIRST YEAR OF LIFE IN YELLOW PERCH[1]

LEE A. FUIMAN

ABSTRACT

Myomeres (preanal and postanal) and vertebrae (precaudal and caudal) were counted on larvae of one year class from southeastern lake Michigan. No variation was associated with growth. Apparent differences in postanal and total myomere number between mid-May and early July samples were explained by recognizing the presence of two cohorts spawned at different times in the same year. The early cohort exhibited fewer postanal and total myomeres probably because of higher incubation temperatures. Vertebra number did not vary from August through October. Total vertebra distributions corresponded with total myomere (minus one) distributions of the early cohort. Myosepta remained intact during hypural formation thereby maintaining the correspondence between myomere number and vertebra number. This correspondence appears to persist in groups of fishes with other vertebral modifications (e.g., Weberian vertebrae and multiple ural centra). The variance of myomere distributions in larvae is less than that of vertebrae in juveniles, indicating possible selection for a particular number of body segments during the larval period.

INTRODUCTION

Myomere number is perhaps the most commonly used quantitative character for identification of fish larvae. The nature of vertebra formation suggests an easily predictable number of myomeres for a larval form when the vertebra count for adults is known. Unfortunately, the correspondence reported in the literature between myomeres in larvae and vertebrae in adults is somewhat less than perfect (cf. Snyder 1979). Among catostomids, I have found (unpublished data) that total myomeres for any of eight species wholly include and extend beyond the reported range of total vertebrae (Weberian vertebrae included). This discordance introduces the following study which investigates natural variability in myomere number with respect to size, time of year, and caudal fin formation for a representative bony fish, the yellow perch (Perca flavescens). The developmental and empirical relationships between myomere and vertebra number are discussed.

METHODS

Yellow perch was chosen for this study because its identification in field samples was not dependent upon myomere counts and because its spawning season did not span a great length of time (long spawning periods might yield thermally induced myomere variation). Also, they are particularly abundant in field samples from Lake Michigan.

Specimens were sampled from a single year class of a population in southeastern Lake Michigan near Port Sheldon, Michigan. Details of the collecting site were given by Jude et al. (1978). Larvae were sampled with a benthic fish-larvae sled (described by Yocum and Tesar 1980) and a 0.5-m diameter, nitex plankton net of 363 μ mesh. Samples containing yellow perch larvae were taken in mid-May and early and mid portions of June and July, 1980. Juveniles (young of the year) were sampled with a semi-balloon, nylon otter trawl having a 0.63-cm bar mesh innerliner, once each during mid portions of August, September, and October, 1980. Larvae were fixed in the field in 10% formalin buffered with sodium borate and later transferred to 5% buffered formalin. Juveniles were frozen in the field, thawed, then preserved in 10% formalin.

Preanal, postanal, and total myomeres were counted with the aid of polarizing filters (essential for accurate counts). Preanal myomeres included all segments whose bordering myosepta were at least partly anterior to the anus, including one segment anterior to the first myoseptum. Postanal myomeres were all segments posterior to the preanal myomeres, including a urostylar segment. The urostylar myomere was included because standard vertebra counts (Hubbs and Lagler 1958) include a hypural (urostylar) centrum. To count vertebrae in juveniles, the right side of the body was dissected away and whole specimens were stained with Alizarin Red S according to the method described by Taylor (1967). Precaudal (abdominal) vertebrae were those with pleural ribs, including any with partially branched ventral processes. Caudal vertebrae were those with unbranched hemal spines. There were no specimens with fused, or otherwise abnormal, vertebrae.

Statistical comparisons of myomere and vertebra distributions were made with nonparametric tests. The median test compared locations of distributions and the Kolmogorov-Smirnov and chi-square for goodness of fit tests compared shapes of distributions (Conover 1980). These tests were chosen because of a concern for lack of normality in the highly peaked distributions and because of the discrete nature and small range of variability of the data. Stated differences in medians or distributions reflect test results at $P < 0.05$ with a two-tailed alternative hypothesis.

RESULTS

Myomeres were counted on 75 larvae ranging from 4.9 to 15.0 mm TL (Fig. 1). Total myomere number did not correlate with total length (correlation coefficient, $r_s = 0.013$), indicating no linear relationship between the variables. Sample sizes for June and mid-July were too small to make inference upon and were excluded from further analyses. The 58 remaining larvae were collected during mid-May and early July. Medians and distributions of preanal myomeres were not different between the two sampling periods. The median for postanal myomeres in mid-May was not different from that in early July; the distributions were different. Medians and distributions of total myomeres were different between the two sampling periods.

Vertebrae were counted on 71 juveniles ranging from 39 to 96 mm TL (Fig. 2a-c). There was no linear relationship between vertebra number and total length ($r = 0.002$ to 0.083). Medians and distributions of vertebrae were not different over the three monthly samples. For simplicity, vertebra distributions for all months were combined (Fig. 2d).

The combined total vertebra distribution was compared with the total myomeres (minus one) distribution of mid-May and of early July (Fig. 3). Medians were not different for mid-May, but were different for early July. Both tests for differences in shape of the distributions were significant for the July comparison, partly a result of the difference in medians. However for the May comparison, the Kolmogorov-Smirnov test was not significant while the chi-

[1]Contribution No. 318 of the Great Lakes Research Division of the University of Michigan.

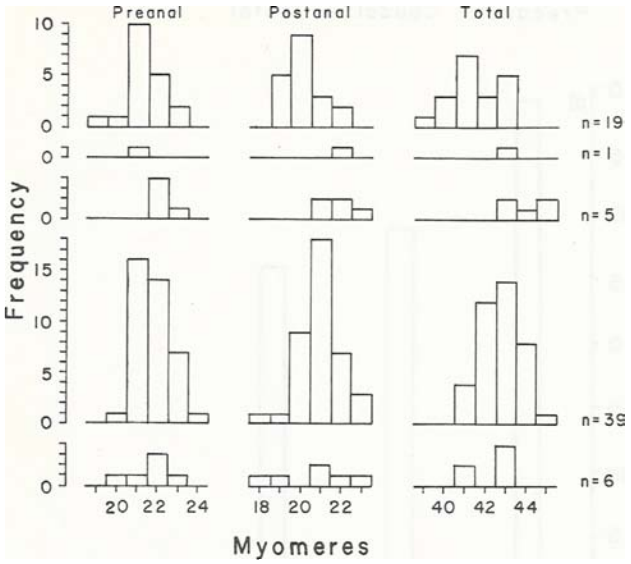

Fig. 1. Frequency distributuions of myomeres for larval yellow perch collected in mid-May (a), early June (b), mid-June (c), early July (d), and mid-July (e).

square test was. In order to meet the criteria of the chi-square test (see Conover 1980), several categories were pooled before analysis, resulting in decreased power of the test. Consequently, the soundness of the conclusion based on this test was reduced. The results of the Kolmogorov-Smirnov test therefore are more reliable. Since the medians did not differ but the distributions did, a test of equal variances (Mood test, Conover 1980) was performed. The results indicated a significant difference among the variances.

DISCUSSION

The ontogeny of teleostean vertebrae illustrates that usually there is not one centrum within each myomere, but rather parts of two centra. The following summary of vertebra development is condensed from Balinski (1965) and Zug (1971) acknowledging Lauder's (1980) insights. During organogenesis the embryo is metamerically arranged into somites. The myotome (myomere) and sclerotome are parts of a somite. Sclerotomes form a continuous, nonsegmented sheath (perichordal tube) around the notochord which later provides the cartilaginous material for formation of vertebrae. Sclerotome concentrates along the myosepta to form neural and hemal arch anlagen. As centra are formed they become segmented usually within each myomere, so that each centrum is connected to two consecutive myomeres. The result is a necessary, exact correspondence of neural and hemal arches with myosepta, and a spurious (or secondary) correlation of centra with myomeres. However, in most teleosts centra usually are intersegmental (Lauder 1980), thereby maintaining a correspondence of myomeres with vertebrae. Myomere number will consistently exceed vertebra number by one in monospondylous fishes, including those with more than one ural centrum. (Geometry requires that n+1 segments result from n parallel partitions within an object). However, many primitive (non-teleost) fishes (e.g., gars) are diplospondylous in the caudal region, thereby reducing the ratio of myomeres to vertebrae.

Analyses of variation in myomere and vertebra number gave no unexpected ultimate results. Myomeres and vertebrae did not change with total length because the number of segments is determined and attained during the embryonic period in most fishes (Fahy 1976, Taning 1952). Few fishes continue to develop myomeres after hatching.

The apparent effect of sampling date on postanal (and total myomere number was not anticipated. However, the significant difference between the distributions of mid-May and early July reflects two cohorts of a single year class spawned at different times. Jude et al. (1978) first hypothesized the presence of these cohorts based on length-frequency data of larvae collected at the same site. They suggested that the earlier cohort was spawned in a small lake (Pigeon Lake) adjacent to Lake Michigan which warms rapidly in spring. In 1980 (this study) recently hatched larvae (5 to 6 mm TL) were collected in early May in Lake Michigan when Pigeon Lake water was between 15 and 17 C, whereas in Lake Michigan the nearshore bottom (where incubation would take place) was first approaching 8 to 11 C, the usual temperature range at which spawning begins (Scott and Crossman 1973). At those temperatures in Pigeon Lake incubation should have been brief, about 10 days (Hokanson and Kleiner 1974). Larvae collected in May presumably represented those spawned in Pigeon Lake, or other warm inland areas, which washed out into Lake Michigan proper. Spawning may have just begun in Lake Michigan proper at this time. Only six larvae were collected in June because most of the early cohort had grown to a size not vulnerable to the sampling gear. Two of the five taken in mid-June were recently hatched, indicating the presence of a second, later cohort. Early July samples contained the most larvae and all sampling periods reflected equal collecting effort. Bottom temperatures in Lake Michigan ranged from 8 to 12 C in mid-June when the second cohort was hatching. This low temperature should have extended the incubation period to approximately 18 to 28 days (Hokanson and Kleiner 1974), suggesting, again, a spawning time during May in Lake Michigan. Insufficient numbers of larvae were collected after mid-July for analysis. This, again, was likely due to growth beyond a size at which they were vulnerable to the gear.

The difference between the incubation temperatures for the early and late cohorts is probably responsible for the observed differences in postanal and total myomere number. (Resh et al. [1976] invoked this argument to explain similarly unexpected results of vertebra counts. However, the fact that their data include possibly several populations and year classes makes proof of this hypothesis difficult). Hubbs (1924), and many others since, have found an increase in mean meristic elements in colder waters (Jordan's rule). Unlike most others, the present example shows a (probably) thermally induced phenotypic difference in a natural situation, within a very small geographic area, and within a single year class of a (probably) single genetic stock. Additionally, the observation that environmentally induced variability was manifested in caudal segments reiterates the high variability in posterior elements observed by Hubbs (1924) for vertebrae and by Fuiman (1979) for myomeres.

The correspondence of total myomeres (minus one) with total vertebrae (Fig. 3) identifies the juveniles collected in August (39 to 61 mm TL), September (61 to 84 mm), and October (72 to 96 mm) as members of the early cohort. A single, 59-mm juvenile taken in October possibly signaled the approach of the late cohort to a size which was vulnerable to trawling.

Correspondence of total myomeres (minus one) with total vertebrae, and lack of change with posthatching growth does not allow for loss of myomeres or myosepta in the process of formation of the hypural complex. Larvae with a completely formed caudal fin exhibited myomere counts on or near the mode for their sampling period. Further, dissection of adult yellow perch showed a distinct, final myoseptum on the ural centrum followed by a single myomere which covered the structural base of the caudal fin. Within an individual, the number of myosepta exactly equalled that of vertebrae and of myomeres (minus one).

These findings may be extended to most other teleosts. Vertebral modification associated with the Weberian ossicles of ostariophysians does not alter myoseptum or myomere number. Inclusion of four Weberian vertebrae is necessary in order for total myomere (minus one) distributions to correspond with total vertebra distributions (unpublished results). Further, I have not found myomere number to change with total length in any of

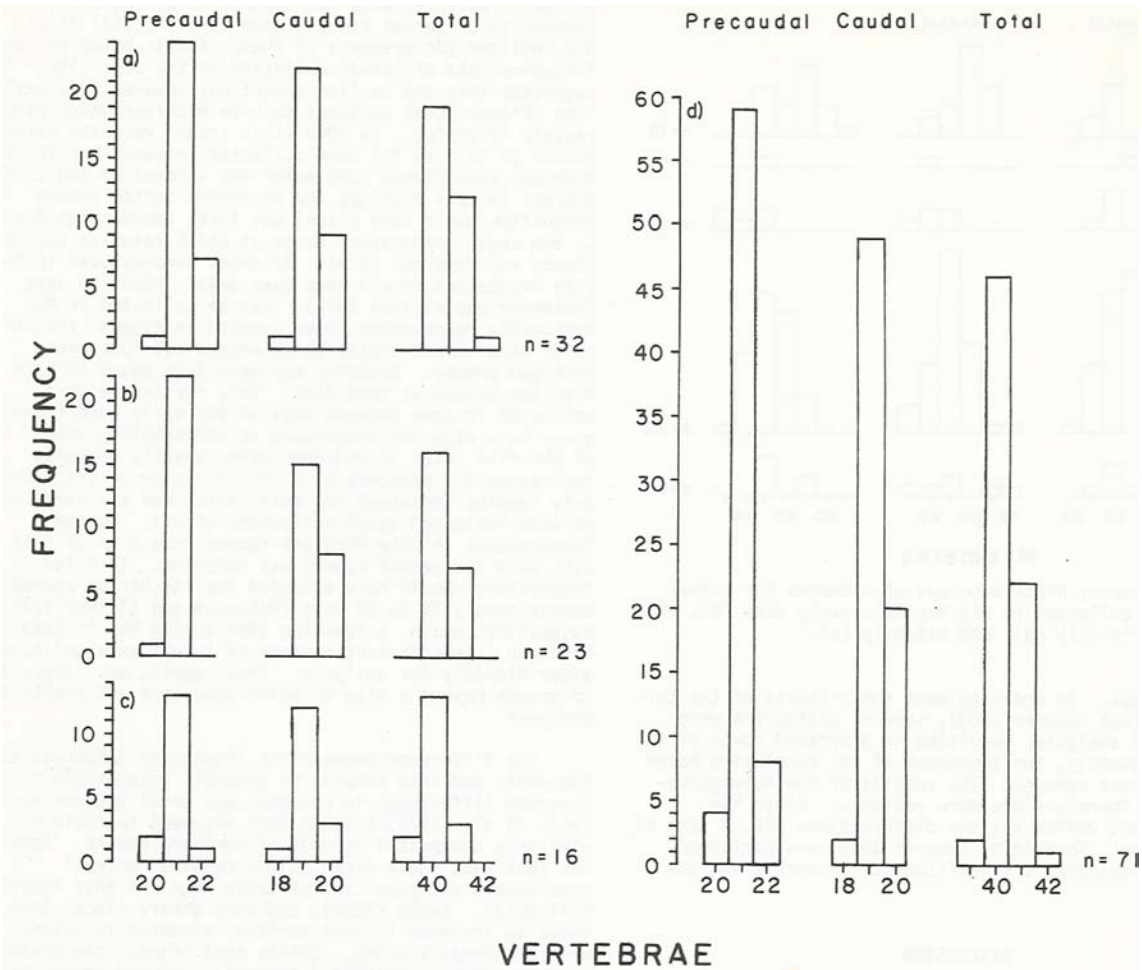

Fig. 2. Frequency distributions of vertebrae for juvenile yellow perch collected in August (a), September (b), October (c), and all months combined (d).

12 North American cyprinoid fishes. More primitive teleosts often incorporate several vertebrae into caudal fin supports (ural centra). This does not appear to modify the number of myosepta. Myosepta matched total vertebrae in dissected adult lake trout (Salvelinus namaycush).

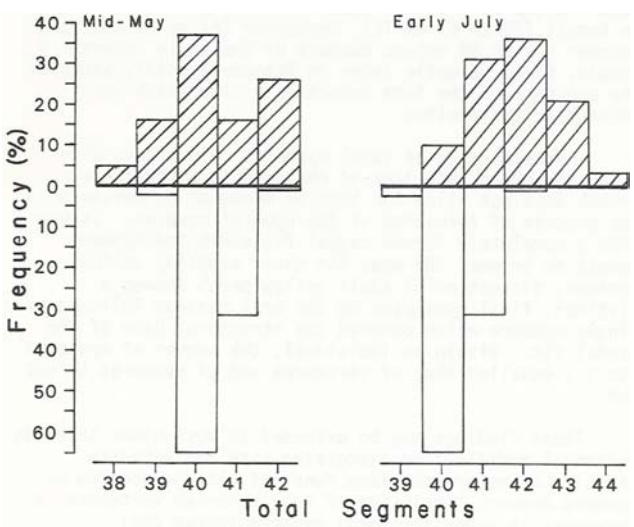

Fig. 3. Relative frequency distributions (as % of sample total) of total myomeres (minus one) (shaded histograms) and total vertebrae (open histograms) for yellow perch.

Taxonomic implications are clear. Myomeres are potentially good characters because they do not change during posthatching stages. They can be counted easily and accurately with transmitted light and polarizing filters, until the opacity of the specimen obscures them (usually late in the larval period). Preanal myomere number is remarkably resistant to environmental modification and its elements are unambiguous (as the final few postanal myomeres may be). Further, total myomere number (minus one matches vertebra number, for which data are abundant, except that vertebrae tend to display less variability. This allows for a confident prediction of myomere number for species in which it is not known.

Descriptions of larval fishes are published frequently wherein myomere data are divided among sizes or developmental phases. This often needlessly reduces the accuracy of the portrayal of the true distribution in a situation where there are often too few specimens to have their statistics divided among three or more size classes. Such a presentation might be useful as a taxonomic tool in itself for a species in which the anus migrates. However, this occurs infrequently.

The discordance among literature reports of myomeres and those of vertebrae for a species probably results from several circumstances. Incubations temperature is an important modifier of vertebra number and as such must be considered when reviewing results based on laboratory-reared specimens. Differences in definition of, and tools for making, myomere counts are contributing factors.

Segments can be overlooked easily without the use of polarizing filters. For example, many of the counts reported by Fish (1932) were several units fewer than modern counts of myomeres or vertebrae, perhaps because of her lack of necessary equipment. Most authors omit the most anterior and the urostylar segments when counting myomeres, further contributing to error. As a result of the comparisons made in this study, I recommend that these segments be included in future accounts since they are, indeed, muscle segments.

Beyond investigator error, there is a real difference in variance between myomere and vertebra distributions, there being less variance in vertebrae. This observation is strongly suggested by the data and tests presented here (Fig. 3) and by unpublished data for several other species. This difference suggests that some individuals may be selected for during the first summer of life. This selection may favor a trait not directly related to vertebra number but somehow linked with it; or some optimal number of body segments may confer greater fitness to an individual (such as enhancing locomotory capabilities, as suggested by Lindsey 1978). Bailey and Gosline (1955) suggested that there is, indeed, environmental selection for a certain number of vertebrae. Perhaps this selection is operating during the larval period.

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INFLUENCE OF BACKGROUND COLOR AND INTENSITY OF ILLUMINATION ON MELANOPHORE EXPANSION IN LARVAL FISH

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Alan H. Mansfield

ABSTRACT

Larval and juvenile spotfin shiners (Notropis spilopterus) were exposed to three levels of illumination and the background colors of white, tan and black. These conditions were maintained for 1 to 4 weeks, at which time the fish were removed and examined under a dissecting microscope. Melanophores on a defined region of the head were counted and their condition assessed as expanded, intermediate or contracted. An index of expansion was calculated by subjectively assigning values of 1, 2 and 3 for each contracted, intermediate and expanded melanophore, respectively. These values were averaged for each individual to obtain a measure of expansion independent of the total number of melanophores. Results indicated greater melanophore expansion over darker backgrounds than lighter backgrounds. This relationship was more pronounced under bright illumination than under dim light. With respect to illumination, melanophores appeared to expand slightly more under dim illumination than bright. Under bright illumination, indices of expansion were 1.7 over white background, 2.1 over tan and 2.5 over black; for moderate illumination, 2.3 over white, 2.3 over tan and 2.5 over black; and for dim illumination 2.1 over white, 2.3 over tan and 2.5 over black. Analysis of variance showed that background color was a significant factor for melanophore expansion, but intensity of illumination was not significant.

INTRODUCTION

Little work has been done on the melanophore response of larval fishes to differing regimes of light intensity and background coloration. Previous studies of pigmentation changes in fishes have dealt with the amount or distribution of pigment under various conditions in adult fishes, but not physiological pigment changes in larval or juvenile fish (Sumner 1940a, Fujii 1969). It is known that the apparent shape of chromatophores is altered by the movement of pigment granules within the cell (Giese 1979). Movement to the cell periphery produces an "expanded" chromatophore and movement of pigment to the center produces a "contracted" chromatophore, although the cell itself does not change shape. In this paper these processes will be referred to as expansion and contraction although these terms are not precise. Movement of pigment granules is generally referred to as physiological pigment change, while increase in number of chromatophores or amount of pigment is designated as a morphological pigment change (Sumner 1940a).

Individual chromatophores can be observed clearly at early life history stages of fish and chromatophore condition assessed as expanded or contracted. In particular, melanophores are easily observed, because black pigment is more conspicuous than other colors. Pigmentation changes have been attributed to changes in background color or brightness and to intensity of illumination (Sumner 1940a; Jenkins 1969; Faber 1980). Consequently, we assessed the effects of those two factors on melanophore expansion in one species of larval fish.

METHODS

Two adult spotfin shiners, Notropis spilopterus (Cope), were collected from Honey Creek about 100 m from its junction with the Huron River, Washtenaw County, Michigan. These shiners spawned on the undersides of rocks in a 110-liter aquarium from 20 June to 10 August, 1980. All larvae studied were offspring of these two fish, minimizing genetic variation. Rocks with attached eggs were moved to another aquarium with water from the original. Larvae were reared to the metalarval phase (Snyder 1976) and some to juveniles; all had numerous melanophores. Prior to the experiment, larvae were kept in an aquarium with sand-colored gravel in a room with indirect sunlight and a few hours per day of artificial light.

Mortality prevented the use of larvae all the same age in the experiment. Five fish were stocked in each tank but data from only four fish were analyzed. Larvae were from five different spawnings which hatched from 12 July to 18 August. Thus, each tank contained larvae and juveniles that ranged in age from 3 weeks to 2 months after hatching.

Nine 4.9-liter transparent plexiglass tanks were filled to a depth of 7 cm with water from the original aquarium. The tanks were prepared with background colors of white, tan and black by covering the outsides of the tanks with colored paper. These were arranged in three groups with one background of each color; each group was subjected to a different light intensity by use of two fluorescent lights of different intensities and a box to shade one group. Light meter readings at the middle of the water column were 72.0 microeinsteins (me)/m^2/sec (bright light), 21.0 me/m^2/sec (medium illumination) and 0.1 me/m^2/sec (shaded tanks). The photoperiod provided was 12 hours of light daily.

Examination of the larvae was begun after 7 days. Observations were always made at the end of the day to maximize effects of the factors studied. It was not possible to make all observations at one time; 3 weeks were required to complete the study. At any given time, larvae from several tanks were examined, rather than completing one tank before beginning another. In this way bias due to length of time larvae were in the tanks was reduced. Each larva was examined under a binocular microscope in a small amount of water until activity slowed due to lack of oxygen. No rapid changes could be seen in melanophore expansion during examination. The melanophores on the occipital and opercular regions of one side of the head were counted. In addition to counting, the melanophores were subjectively assigned an index related to the dispersal of pigment within. A melanophore which appeared as a simple round spot was said to be contracted (index = 1); one with protrusions equal to or less than the diameter of the center of the cell (a stellate melanophore) was called intermediate (index = 2); and a melanophore with protrusions longer than the diameter of the center (a reticulate melanophore) was called expanded (index = 3) (terminology from Giese 1979). Most larvae examined had melanophores in more than one of these conditions, so the number of melanophores in each condition was recorded for each individual. After examination, larvae were removed from the study tanks. A mean index of expansion was calculated for each specimen, and the mean for each tank. Juveniles tended to have greater absolute numbers of melanophores, and some juveniles from each exposure were examined to eliminate any bias introduced by age differences. The values thus obtained were used to test the significance of the two factors, light intensity and background color.

A two-factor analysis of variance (Remington and Schork 1970) was performed to compare the effects of background color and light intensity. Bartlett's test (Steel and Torrie 1960) and frequency plots of the indices and residuals showed that assumptions of normality and homogeneity of variance were not seriously violated.

RESULTS

Melanophore expansion was greater in fish exposed to a dark background than in those exposed to lighter backgrounds (Table 1). Analysis of variance showed this

relationship to be significant at the 5.0% level (Table 2). Light intensity, however, did not significantly affect melanophore expansion at the 5.0% level. Since illumination and background color were independently controlled, no significant interaction between factors was expected; this proved to be the case. Statistically, then, the response to background color did not vary for each illumination level.

Table 1. Mean melanophore expansion indices for spotfin shiner larvae exposed to three illumination levels and three background colors.

	Backgrounds		
	White	Tan	Black
Bright illumination	1.7	2.1	2.5
Moderate illumination	2.3	2.3	2.5
Dim illumination	2.1	2.3	2.5

Table 2. Summary of analysis of variance on spotfin shiner melanophore response to background color and intensity of illumination.

Source	df	Mean squares	F
Illumination	2	8.25	2.23
Background	2	24.45	6.61 *
Interaction	4	0.28	0.07
Error	27	3.70	
Total	35		

* Significant ($P < 0.05$)

Considering only mean values for each tank (Table 1), the difference in melanophore expansion over different backgrounds was more pronounced in bright illumination; the indices displayed a wider range for bright light than for medium or dim. In addition, for white background only, melanophores appeared to expand somewhat more in dimmer illumination. However, neither trend was consistent, nor significant in the analysis of variance. The only significant factor in the investigation was background color.

Although both larvae and juveniles were included in this study, there was no apparent trend in the expansion index with age. Juveniles and larvae were similar with respect to number of expanded melanophores. Behavioral interactions between juveniles and larvae appeared to be minimal, suggesting that melanophore development in larvae was not affected by the presence of juveniles.

DISCUSSION

The idea that background color is the most important determining factor for pigmentation changes in fishes was substantiated by our study. One important adaptive pressure on young fishes is to visually merge with the background and thus avoid predation. Therefore those individuals which are least conspicuous have a selective advantage. In this respect, intensity of illumination may not be expected to have much effect on pigmentation change, as long as the fish matches its background. As melanophores of larvae expand, their macroscopic appearance is darker, so a larva with expanded melanophores blends with a dark background.

If the fish and background receive dim illumination, the stimulus and adaptive advantage for chromatophore response are reduced and background color would not be expected to affect melanophore expansion as much. In this study some differences in expansion under dim illumination were observed, but were not statistically significant.

The more pronounced expansion of melanophores under dim illumination than bright illumination, over white background in our study, was not statistically significant, but may represent a trend. In dim illumination, background color may be perceived as darker than its true color, causing melanophore expansion. Experimentally blinded fish also exhibit expansion of melanophores (Pickford and Kosto 1957), which may in similar manner indicate perception of the background color as dark when it cannot be seen by the fish. Fishes kept in a dark container for a long time develop increased melanin content of the skin and a greater number of melanophores (Odiorne 1957), a morphological color change consistent with the physiological color changes discussed here.

In fish, control of chromatophores is both by nerve impulses and by hormones (Fingerman 1970, Abbott 1970). Fujii (1969) reviews several hypotheses concerning the mechanisms of pigment granule movement: gelation and solation of cytoplasm, electrophoresis, and function of microtubules as cytoskeletal elements. Melanocyte-stimulating hormone (MSH) disperses melanin in melanophores in the presence of sodium (Na) ions. Several pituitary hormones affect dispersion and aggregation of pigment in teleost fish; however, in many species melanophores are primarily controlled by the autonomic nervous system (Fujii 1969). Incomplete understanding of the complexity of innervation and hormone interactions prevents our relating these mechanisms to melanophore responses in our study.

Previous studies of the effects of illumination on pigmentation are inconclusive. Sumner (1940b) subjected adult guppies, Poecilia reticulata Peters, to four intensities of illumination and two background colors. Melanophore number, rather than melanophore expansion, was used as a measure of pigmentation change. The results for illumination were not statistically significant, while more melanin was present in those fish kept on a black background than on gray. Sumner's results are consistent with ours.

A more recent study on larval golden shiners, Notemigonus crysoleucas (Mitchill), included observations on dorsal pigmentation (Faber 1980). Larvae collected from an unshaded surface area of a lake had more expanded melanophores than larvae from a shady area. Faber stated that this was an apparent contradiction of Sumner's (1940a) observations that dark background or low albedo causes an expansion of melanophores and light background causes contraction. Apparently the sunlight and its subsequent diffusion in the water produced a background color independent of the substrate, since Faber did not mention a substrate color change. He speculates that an extremely high irradiance of sunlight may cause the contradiction, contrary to our results. Perhaps the shaded area received insufficient light to stimulate an expansion response in the melanophores. There may be other factors operating as well, since many variables are present in the field that are not present in the laboratory. Ultraviolet light, temperature and pH have all been shown to affect melanophore response (Fujii 1969).

Studies of melanophore expansion are relevant to taxonomy. Frequently keys to identification of larval fish use characteristics such as the presence of stellate or dendritic malanophores. Bracken and Kennedy (1967) stated in a taxonomic study that melanophore expansion does not vary in larval fish as much as in adults; however, this may be because larvae are characteristically found in or near the spawning habitat and their degree of melanophore expansion is a result of the background color. It has been demonstrated in this study and by Faber (1980) that larval fishes of a given species may show varying degrees of melanophore expansion under different conditions. Therefore, shape of melanophores is not always a reliable character to use for identification

of larval fishes.

Preservation of larvae further confounds use of pigment as a taxonomic character. Although we examined live larvae for this study, some were preserved after examination. Differences in melanophore expansion continued to be visible for several weeks in preserved specimens, but pigment gradually faded until melanophores appeared similar between individuals. In larvae preserved in formaldehyde, pigment is often virtually invisible after several years.

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EARLY DEVELOPMENT OF THE GENUS ICTIOBUS (CATOSTOMIDAE)

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The early development of the three species of Ictiobus is described from larvae reared under artificial conditions. Hatching sizes of Ictiobus ranged from 5.0 to 6.3 mm. Diagnostic characters for larvae of the genus Ictiobus include a low number of preanal myomeres (26 to 31, very rarely 32), presence of occipital pigment, hatching size smaller than most catostomids, smaller size at particular stages of development, long dorsal fin base, and gross body morphology. Additionally, Ictiobus lacks distinctly elliptical eyes and a flattened head as found in the genus Carpiodes and has a more defined midventral line of melanophores than in Carpiodes. Despite acquisition of meristic and morphometric data, and observations on gross morphology, sizes at stages of development, and pigmentation patterns, no single definitive character or combination of definitive characters for identification of early buffalo larvae to species was attained.

INTRODUCTION

Buffalofishes, members of the catostomid genus Ictiobus, comprise a major portion of the food fishes consumed on local markets in the central and southeastern United States and are exported to markets in Los Angeles, Chicago, and New York. Despite past efforts to promote the buffalofishes in fish husbandry, the major source of these fishes remains commercial trammel netting in lakes, reservoirs, and major tributaries of the Mississippi River drainage. With the ever-increasing multiple use of these major river systems for industrial or power plant siting and disposal of municipal wastes, the potential for adverse effects on such a major fishery is great.

Early life history stages, particularly the pelagic newly hatched larvae of buffalo, are susceptible to entrainment or transport into areas impacted by industrial heat or wastes. Inability to identify particular taxa of ecologically related species has hampered investigations of larval ichthyofauna. The ictiobine subfamily, comprised of the genera Ictiobus (buffalofishes) and Carpiodes (carpsuckers), is no exception. This report provides descriptions of the early development of the three species of Ictiobus with comparisons to Carpiodes and other catostomid genera.

METHODS AND MATERIALS

Broodstock of the bigmouth buffalo (I. cyprinellus) were seined from 0.1 and 0.2 ha holding ponds at the Fish Farming Experimental Station, U.S. Fish and Wildlife Service (USFWS), Stuttgart, Arkansas, where they had been overwintered. The buffalo originally were trammel netted from the Arkansas River in Arkansas, as were the black buffalo (I. niger). Adult smallmouth buffalo (I. bubalus) were similarly captured from the Cache River and Bayou DeView in Arkansas.

All fish were injected intraperitoneally with 0.2-mg carp pituitary per 0.42 kg (1b) of body weight. Spawning methods of Walker and Frank (1952) were utilized. Incubation of eggs was in McDonald jars supplied with well water at 20 C. Newly hatched larvae were held in stainless steel troughs supplied with well water until transfer to Norris, Tennessee, in plastic bags with oxygenated water. At the Norris wet laboratory facilities of the Tennessee Valley Authority (TVA), larvae were held in 83 x 70 x 10 cm white plastic trays supplied with aerated spring water at 15 to 19 C. Egg yolk and finely ground trout chow were fed to the larvae daily from hatching to 10 days posthatching. Thereafter newly hatched brine shrimp (Artemia) replaced the egg yolk.

Young buffalofishes were preserved daily in 10 percent formalin and then transferred to 5 percent formalin buffered to pH 7.0 with ammonium hydroxide. The series were cataloged into the reference collection of the Larval Fish Identification and Information Center, TVA, Norris, Tennessee.

A Wild M-5 stereomicroscope equipped with an ocular micrometer and polarizers was utilized for recording morphometric and meristic data. Characters examined were: total, standard, preanal, predorsal, snout, and head lengths; head depth; body depth at the anus; eye diameter; head width; yolk sac length and depth at hatching; numbers of preanal and postanal myomeres; and numbers of fin rays. Morphology and pigment patterns were described. Pigmentation development was so similar among the three species that while only a description for the smallmouth buffalo is presented herein, it is also applicable to the bigmouth and black buffalos. Definitions of characters follow Yeager (1980). The method of counting myomeres follows Hogue et al. (1976). Terminology utilized follows Snyder (1976). Unless otherwise stated, all measurements mentioned are total lengths. Drawings were made with the aid of a camera lucida. Fin ray counts and squamation were noted by staining specimens in a solution of 0.2 to 0.3 g l^{-1} methylene blue in distilled water.

SPECIES DESCRIPTIONS

Smallmouth Buffalo, Ictiobus bubalus (Rafinesque).--The series consisted of 40 eggs and 430 larvae and juveniles. Morphometric or meristic data (Tables 1 and 2) were recorded from 138 specimens. Mean egg diameter was 2.3 mm (5 eggs) and ranged between 2.3 and 2.4 mm. The total lengths of cultured smallmouth buffalo ranged from 5.0 to 32.7 mm.

Protolarvae ranged from 5.0 to 9.5 mm (Figures 1a to 1c). Mean total length of 20 specimens at hatching was 5.8 mm (range 5.0 to 6.3 mm). Modal numbers and ranges (in parentheses) of preanal, postanal, and total myomeres (35 specimens) were 30 (28-31), 8 (6-9), and 38 (35-39), respectively.

At hatching the head was strongly to moderately decurved. The yolk sac was club-shaped. Yolk material was pale yellow and no oil globules were present. Auditory vesicles were present (distinct only in dorsal view) and otoliths were visible under polarized light. The oral pit was subterminal and ventral. Eyes were circular to slightly elliptical. The median finfold originated dorsally at the 10th to 13th preanal myomere, was continuous around the urostyle, and extended forward ventrally to the anterior portion of the yolk sac. Pectoral buds were present at hatching. Pectoral buds became paddlelike by 6.5 mm (1 day posthatching) and remained so throughout the protolarval phase. Opercles were not developed at hatching and covered only the first two gill arches on late protolarvae (9.5 mm). By 6.5 mm (1 day posthatching) the head was no longer decurved, the mouth was open, and the lower jaw was beginning to form. The lower jaw reached the upper jaw by 7.0 mm and the mouth had moved to a terminal position, with the middle of the upper jaw in line with the middle of the eye. By 6.8 mm the gas bladder began to form and the yolk sac was cylindrical. Yolk absorption was complete by 7.5 mm.

Table 1. Morphometric data ($\bar{x}$ as % TL or head length with s.d. in parenthesis) for smallmouth buffalo.

Total Length Range (Mean Standard Deviation)	N	Percent Total Length: Standard Length	Preanal Length	Predorsal Length	Head Length	Body Depth at Anus	Percent Head Length: Head Depth	Head Width	Snout Length	Eye Diameter
5 0 5 6 (5 6 0 22)	10	96 5 (0 48)	74 6 (3 85)	40 7 (2 6)	16 6 (0 99)	8 1 (0 28)	72 9 (6 0)	62 8 (4 2)	23 3 (3 3)	41 7 (4 2)
5 0 5 8 (5 5 0 25)	10	95 1 (1 0)	73 8 (4 0)	39 5 (2 0)	17 3 (0 64)	8 5 (0 43)	72 4 (2 6)	59 2 (4 3)	21 8 (4 0)	38 6 (3 2)
7 0 7 7 (7 4 0 23)	10	94 5 (0 46)	70 7 (0 59)	37 9 (1 10)	17 4 (0 44)	7 9 (0 48)	68 2 (3 86)	58 7 (3 34)	9 2 (1 23)	38 1 (1 74)
8 1 8 0 (8 5 0 27)	4	94 5 (0 18)	73 3 (0 42)	38 8 (0 90)	18 5 (0 70)	9 8 (0 83)	54 5 (3 64)	61 0 (3 16)	8 8 (0 82)	35 7 (3 46)
9 5	1	94 7	73 9	39 1	19 4	9 4	55 8	65 8	8 6	36 9
10 0 10 3 (10 2 0 29)	3	91 9 (1 54)	73 3 (0 82)	40 7 (1 00)	20 5 (0 63)	10 6 (0 58)	58 0 (1 96)	61 2 (0 67)	13 4 (0 35)	37 0 (1 35)
11 5 12 0 (11 8 0 27)	6	89 4 (1 46)	71 5 (3 84)	40 3 (1 98)	20 2 (0 59)	10 3 (0 37)	63 1 (2 85)	62 C (1 59)	14 3 (0 84)	36 9 (1 10)
12 2-12 8 (12 5 0 26)	3	88 7 (0 23)	71 4 (1 00)	39 9 (1 12)	20 4 (0 42)	10 3 (0 79)	62 5 (1 56)	61 5 (0 90)	15 1 (0 93)	34 9 (0 90)
13 1 13 8 (13 5 0 33)	4	86 4 (1 73)	69 3 (1 42)	39 2 (0 95)	20 2 (0 73)	10 4 (0 38)	54 5 (1 89)	61 0 (1 18)	15 0 (1 25)	34 5 (1 55)
14 3-14 7 (14 4 0 19)	6	84 9 (0 23)	68 4 (1 88)	40 1 (0 84)	21 7 (0 55)	11 3 (0 41)	51 9 (1 79)	60 0 (1 46)	15 6 (0 40)	36 7 (0 85)
15 3, 15 8 (1 6 0 22)	6	83 7 (1 21)	67 8 (1 64)	40 1 (0 98)	22 0 (0 52)	11 8 (0 59)	52 6 (1 53)	60 0 (2 04)	17 3 (0 63)	33 5 (0 84)
16 2-16 8 (16 5, 0 29)	5	83 0 (0 82)	68 4 (1 26)	39 3 (1 18)	23 0 (0 25)	12 7 (0 73)	51 7 (1 82)	59 4 (1 11)	19 5 (1 77)	32 1 (1 05)
17 0-17 9 (17 3, 0 31)	7	81 9 (0 63)	66 2 (1 49)	38 3 (0 68)	22 6 (0 45)	12 3 (0 66)	52 8 (1 60)	60 9 (0 93)	18 5 (0 46)	31 3 (1 29)
18 2-18 9 (18 6, 0 34)	4	81 4 (0 80)	64 8 (0 95)	39 3 (1 34)	25 0 (2 87)	14 3 (0 55)	54 1 (5 24)	56 1 (4 25)	16 8 (2 27)	29 1 (2 48)
19 2-19 8 (19 4, 0 27)	6	80 1 (1 29)	62 2 (1 32)	38 2 (0 78)	23 4 (0 30)	14 3 (0 84)	61 8 (1 38)	58 3 (1 00)	18 6 (1 38)	30 3 (0 84)
20 0-20 7 (20 2, 0 36)	3	78 9 (90 47)	61 9 (0 22)	37 0 (0 31)	22 7 (0 46)	14 0 (0 43)	61 8 (1 74)	58 / (0 99)	20 6 (1 23)	29 3 (1 21)
21 0-21 8 (21 4, 0 37)	5	79 5 (1 06)	62 4 (1 26)	37 8 (1 35)	24 0 (0 45)	14 6 (0 70)	63 4 (2 39)	57 9 (2 00)	21 2 (0 75)	29 6 (0 81)
22 2-22 8 (22 4, 0 18)	10	78 3 (1 33)	61 0 (1 50)	36 8 (1 58)	23 2 (1 04)	15 3 (0 75)	65 6 (2 82)	60 0 (3 12)	21 3 (1 17)	30 0 (1 08)
23 0-23 9 (23 5, 0 32)	10	78 6 (1 35)	60 3 (1 09)	38 0 (1 19)	23 5 (0 72)	15 7 (0 43)	65 7 (2 13)	57 9 (2 46)	22 0 (1 62)	29 0 (1 24)
24 2-24 8 (24 5, 0 17)	6	78 2 (0 71)	60 0 (0 75)	36 9 (1 03)	23 1 (0 42)	15 0 (0 43)	65 1 (1 97)	57 3 (4 17)	22 6 (1 05)	29 / (0 90)
25 2-25 7 (25 3 0 21)	6	79 5 (2 88)	56 9 (0 97)	38 4 (1 13)	23 5 (0 67)	15 4 (0 54)	65 3 (1 99)	58 1 (2 15)	22 6 (0 98)	27 6 (0 74)
26 0-26 7 (26 3 0 28)	6	77 1 (2 08)	59 7 (1 94)	36 9 (1 27)	22 5 (0 74)	15 3 (0 81)	66 0 (2 84)	60 0 (1 49)	23 7 (1 50)	27 0 (1 43)

Table 2. Mode and range for fin rays in juvenile buffalo.

Species	Number of Specimens	Size Range (mm)	Dorsal Fin Rays	Anal Fin Rays	Caudal Fin Rays	Pectoral Fin Rays	Pelvic Fin Rays
Ictiobus bubalus	9	27 5-32 7	28 (24 28)	9 (7 9)	17 (16-19)	16 (15-17)	9 (8-10)
Ictiobus cyprinellas	13	29 4-75 2	25 (22-29)	8 (8)	16 (16-19)	16 (15 16)	10 (10 11)
Ictiobus niger	1	25 7	30	9	18	15	10

Table 3. Morphometric data ($\bar{x}$ as % of TL or head length with s.d. in parenthesis) for bigmouth buffalo.

Total Length Range (Mean Standard Deviation)	N	Percent Total Length: Standard Length	Preanal Length	Predorsal Length	Head Length	Body Depth at Anus	Percent Head Length: Head Depth	Head Width	Snout Length	Eye Diameter
5 5 5 9 (5 7 0 11)	10	96 9 (0 60)	74 9 (1 07)	41 5 (2 99)	16 1 (0 52)	8 2 (0 56)	72 4 (3 09)	64 6 (3 12)	23 3 (1 88)	43 5 (1 93)
6 2 6 8 (6 5 0 25)	10	95 0 (0 73)	71 7 (0 85)	38 7 (1 59)	16 5 (0 64)	7 9 (0 24)	72 1 (2 98)	64 2 (3 65)	17 9 (1 24)	40 6 (1 86)
7 0 7 7 (7 3 0 23)	10	94 3 (0 96)	71 4 (1 54)	37 2 (1 71)	16 9 (0 78)	7 9 (0 63)	57 2 (2 93)	65 2 (2 34)	12 5 (1 38)	40 4 (2 03)
8 0 8 5 (8 4 0 25)	10	93 4 (1 07)	72 1 (1 42)	37 9 (1 57)	18 3 (0 73)	8 9 (0 53)	53 5 (3 93)	65 0 (2 19)	12 4 (1 46)	39 0 (3 15)
9 1 10 C (9 6 0 32)	10	94 3 (0 95)	73 C (1 57)	38 3 (1 16)	17 8 (0 46)	8 9 (0 58)	67 8 (2 85)	65 4 (2 90)	11 8 (1 29)	40 0 (1 75)
10 2 10 8 (10 5 0 26)	10	92 0 (1 34)	71 7 (1 79)	39 3 (1 38)	19 3 (1 19)	10 2 (0 69)	53 5 (2 85)	64 0 (2 64)	14 4 (1 40)	38 1 (1 55)
11 0 11 9 (11 4 0 31)	10	89 2 (1 35)	70 6 (1 23)	38 6 (1 19)	20 5 (0 86)	10 0 (0 53)	60 5 (4 13)	59 9 (3 55)	15 5 (2 93)	34 3 (1 94)
12 0 12 9 (12 3 0 34)	10	90 2 (1 39)	71 5 (1 93)	38 5 (1 06)	19 7 (0 90)	9 9 (0 56)	63 0 (2 81)	61 9 (3 15)	15 0 (2 01)	36 1 (2 19)
13 0 13 9 (13 5 0 30)	10	86 9 (1 72)	70 7 (1 58)	37 3 (4 02)	20 3 (1 28)	10 5 (0 77)	65 5 (5 79)	60 1 (2 46)	16 4 (1 36)	35 1 (2 52)
14 1 15 0 (14 3 0 27)	10	84 4 (2 48)	68 5 (1 81)	39 3 (1 29)	21 5 (1 93)	10 6 (0 71)	63 8 (3 65)	59 3 (3 03)	16 2 (1 19)	34 4 (2 55)
15 1 15 8 (15 6 0 24)	10	82 C (1 80)	66 1 (1 64)	39 5 (0 85)	22 7 (1 05)	11 8 (0 83)	62 7 (2 70)	58 5 (2 35)	18 3 (1 03)	35 7 (1 94)
16 2 16 8 (16 3 0 21)	10	80 4 (3 20)	65 5 (2 73)	39 0 (0 70)	24 4 (2 37)	12 0 (0 90)	50 9 (2 29)	54 9 (3 20)	17 9 (1 55)	28 9 (2 70)
17 0 17 9 (17 4 0 29)	10	79 7 (1 39)	63 3 (1 84)	37 8 (1 60)	24 8 (1 53)	13 1 (0 59)	51 9 (2 87)	57 0 (1 69)	19 2 (1 84)	27 8 (1 81)
18 0-18 7 (18 4 0 26)	7	79 3 (0 72)	62 3 (1 15)	39 3 (3 88)	24 3 (2 00)	13 3 (0 84)	53 1 (1 82)	58 1 (1 77)	19 4 (2 06)	27 8 (2 22)
19 0 19 7 (19 4 0 29)	7	79 3 (1 05)	62 1 (1 72)	37 9 (2 41)	25 4 (1 40)	13 2 (0 43)	51 1 (3 75)	54 8 (2 58)	19 8 (1 65)	16 6 (1 93)
20 1 20 9 (20 6 0 31)	9	78 4 (1 80)	60 9 (1 62)	37 8 (1 12)	25 4 (1 35)	13 9 (0 57)	60 0 (3 84)	54 3 (2 94)	20 5 (1 65)	26 4 (1 83)
21 1 21 9 (21 6 0 33)	6	76 0 (2 11)	58 3 (2 30)	36 4 (0 73)	26 3 (1 30)	14 1 (0 59)	59 0 (2 73)	52 6 (2 82)	20 3 (1 39)	25 4 (1 04)
22 1 22 9 (22 5 0 29)	9	77 1 (1 68)	59 6 (1 85)	37 5 (1 39)	26 8 (0 70)	14 7 (0 59)	61 4 (3 43)	53 7 (2 65)	19 9 (0 94)	25 5 (1 71)
23 1 23 9 (23 6 0 26)	8	77 7 (1 93)	59 7 (1 51)	37 4 (2 09)	27 1 (1 38)	15 5 (1 47)	62 7 (5 51)	56 0 (2 44)	20 4 (1 06)	24 9 (1 20)
24 1 24 8 (24 6 0 31)	6	76 4 (0 53)	58 1 (0 78)	36 9 (0 43)	27 8 (1 21)	14 4 (0 67)	60 1 (2 94)	53 5 (2 81)	21 4 (1 67)	25 4 (1 57)
25 0 25 8 (25 4 0 43)	5	77 5 (1 09)	60 3 (1 15)	37 3 (0 93)	27 4 (1 35)	14 7 (1 37)	59 3 (2 81)	54 5 (3 06)	21 8 (1 24)	24 5 (1 27)
27 5 29 1 (28 0 0 93)	5	78 4 (3 35)	59 5 (2 23)	37 0 (2 26)	27 7 (1 16)	16 1 (1 66)	62 3 (3 53)	55 7 (2 80)	21 6 (0 78)	23 4 (1 75)

The urostyle was angled up slightly by 9.5 mm and an opaque area formed ventrally signifying the onset of caudal fin ray development. The protolarval phase ended with the formation of caudal rays on specimens 10 mm or greater.

Larvae at hatching had darkly pigmented eyes and a diffuse row of melanophores on the yolk sac ventrum. Additional protolarval pigmentation patterns developed in 1 to 3 days posthatching (6 to 7 mm). Dorsal pigmentation consisted of a few melanophores on the snout, 15 to 25 on the head and occiput, and a double row from the nape to the urostyle. A midlateral line of 13 to 15 melanophores was present. Internally, the dorsum of the incipient air bladder and gut was pigmented.

Mesolarvae (Figures 1d to 1f) ranged from 9.6 to 23.1 mm. By 9.6 mm caudal fin rays first appeared and by 10.4 mm the hypural complex was evident. The caudal fin was emarginate by 15.1 mm and the dorsal lobe was slightly longer than the ventral lobe. By 12.8 to 14.2 mm the adult complement of caudal fin rays was present.

Differentiation of the dorsal fin outline was evident by the beginning of the mesolarval phase. The apex was situated over the 17th to 19th preanal myomeres. Dorsal fin rays appeared by 15.1 mm and the adult complement was attained by 22.2 to 23.6 mm. Appearance of initial anal fin rays varied between 15.1 and 18.0 mm. The adult complement was present by 21.0 to 23.6 mm.

By 17.8 to 18.0 mm pectoral fin rays had formed. Ventrolateral folds along the body wall at 12.0 mm were the first indication of pelvic bud formation. By 14.5 mm the pelvic buds were wide-based flaps. Pelvic fin rays appeared by 17.8 to 18.0 mm. At 20.1 mm the only remaining fin fold was along the ventral foregut.

The mouth became slightly subterminal by 15.1 to 17.8 mm and was distinctly so by 19.4 to 20.1 mm. Coiling of the gut began at 16.5 to 18.0 mm. One full coil was present on specimens larger than 18.0 mm. By 12.8 mm the second gas bladder chamber appeared as an anterior diverticulum of the primary chamber, and two distinct chambers were present by 14.2 mm.

An indistinct middorsal row of melanophores, situated between the two rows described on protolarvae, formed and became increasingly concentrated throughout the mesolarval phase. The nape, head, and snout became heavily pigmented. Ventrally, a diffuse row of melanophores extended from the branchiostegal region to the caudal fin. The branchiostegals and gills had scattered melanophores. Scattered melanophores were on the cheek, maxilla, opercle, and along the dorsolateral flanks. The midlateral line of pigment became more distinct and extended from the opercle to the caudal fin base. Caudal fin rays were stitched with melanophores. Progressive pigmentation of fin rays to the distal fin edge occurred on the dorsal, anal, and pectoral fins.

The metalarval phase (Figure 1g) began with the attainment of the adult complement of median fin rays at 22.2 to 23.6 mm. Adult pelvic and pectoral fin ray counts were attained at 23.6 to 26.1 mm and 27.5 to 30.6 mm, respectively. Median fin fold absorption was completed by 28.0 to 30.6 mm. The mouth remained subterminal and ventral throughout metalarval development.

During metalarval development, dorsal pigmentation extended down to or below the midline. The middorsal row of melanophores became more distinct. It extended from the head to the dorsal fin, separated into two rows around the dorsal fin, and was present as a scattered row posteriorly. The double dorsal row became faint. A triangular concentration of melanophores was situated over the midbrain.

Melanophores were spread along the lateral body. The pigmentation graded to less dense from the dorsum to venter of the body. The areas around the base of the pectoral fins, belly, and breast were devoid of pigment. A double ventral row of melanophores was present from the anus to the base of the caudal fin. All median fins were pigmented to the distal edges.

Upon complete absorption of the median fin fold and attainment of the adult complement of rays in all fins, the juvenile period (Figure 1h) began between 27.5 and 30.6 mm. The mouth was subterminal and ventral. Fin ray counts appear in Table 2.

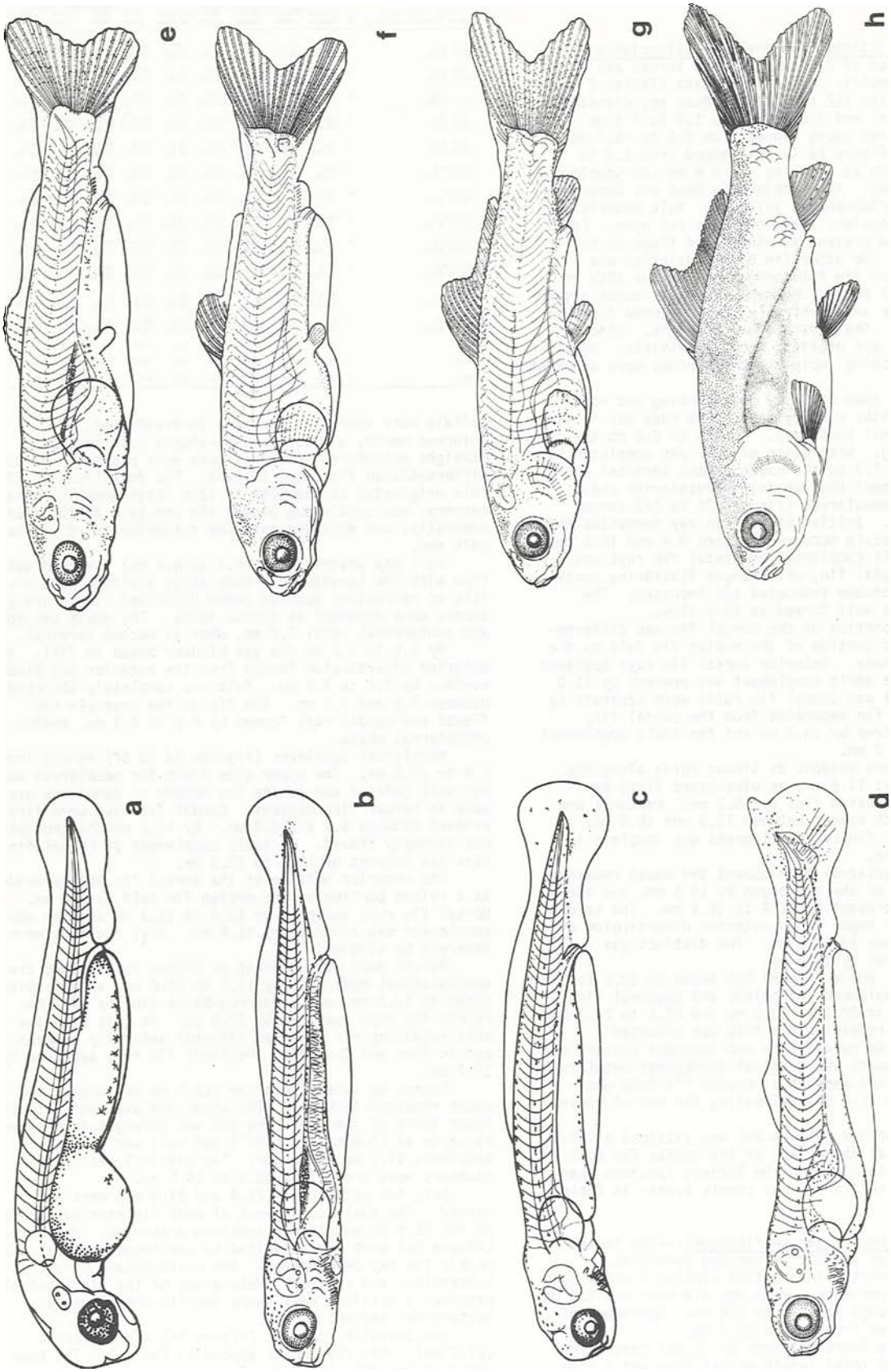

Figure 1. Ictiobus bubalus: (a) protolarva 5.6 mm, (b) protolarva 6.8 mm, (c) protolarva 7.7 mm, (d) mesolarva 10.4 mm, (e) mesolarva 16.2 mm, (f) mesolarva 17.8 mm, (g) metalarva 21.5 mm, and (h) juvenile 29.1 mm

Though more concentrated and dense, melanophore patterns on early juveniles resembled those of metalarval specimens.

Bigmouth buffalo, Ictiobus cyprinellus (Valenciennes).--The series consisted of 578 eggs and 486 larvae and juveniles. Morphometric and meristic data (Tables 2 and 3) were recorded from 212 specimens. Mean egg diameter was 2.0 mm (20 eggs) and the range was 1.9 to 2.1 mm. Specimens of cultured young ranged from 5.0 to 76.3 mm.

Protolarvae (Figure 2a to 2c) ranged from 5.0 to 9.4 mm. Mean length at hatching was 5.6 mm (20 specimens, range 5.0 to 6.3 mm). At hatching the head was decurved over the bulbous, club-shaped yolk sac. Yolk material was pale yellow and granular. The mouth was not open. Pectoral fin buds were present as wide-based flaps on the anterior yolk sac. No other fin differentiation was evident. The median fin fold originated at the 10th to 13th (12th modally) preanal myomere, was continuous around the urostyle to the anus ventrally, and extended forward onto the yolk sac. The urostyle was straight. Otic vesicles were evident but otoliths were not visible. Within a few hours of hatching incipient gill arches were apparent as tissue folds.

The mouth was open by 1 day posthatching and was subterminal. Otoliths were evident. The head was in line with the longitudinal body axis. By 6.5 to 6.8 mm the gas bladder was filling. Absorption of yolk was completed by 6.7 to 7.3 mm. By 7.3 mm the mouth became terminal and remained so throughout the remaining protolarval phase.

Specimens of mesolarvae (Figures 2d to 2f) ranged from 9.4 to 1.3 mm. Initial caudal fin ray formation and flexion of the urostyle occurred between 9.4 and 10.0 mm. By 13.0 mm the adult complement of caudal fin rays was present and the caudal fin, which began flattening posteriorly at 7.5 mm, became truncated and indented. The hypural complex was well formed at this size.

The anterior portion of the dorsal fin was differentiating as a raised portion of the median fin fold by the early mesolarval phase. Anterior dorsal fin rays appeared by 13.9 mm, and the adult complement was present by 21.0 to 23.5 mm. Dorsal and caudal fin folds were separate by 15.3 mm. The anal fin separated from the caudal fin. Anal fin rays appeared by 15.3 mm and the adult complement was attained by 20.3 mm.

Pelvic buds were evident as tissue folds along the ventral body wall at 11.9 mm, as wide-based flaps by 14.6 mm, and narrow-based fins by 15.3 mm. Pectoral and pelvic fin rays both appear between 15.3 and 16.5 mm. Pelvic and pectoral fin ray development was complete in the metalarval phase.

Throughout mesolarval development the mouth remained terminal. Coiling of the gut began by 15.5 mm, and one full gut coil was present by 16.4 to 16.9 mm. The second gas bladder chamber began as an anterior diverticulum of the posterior chamber at 14.2 mm. Two distinct gas chambers were present by 16.6 mm.

The metalarval phase (Figure 2g) began by 22.1 to 22.4 mm. Adult complements of pelvic and pectoral fins rays were attained at 22.1 to 23.5 mm and 22.1 to 24.3 mm, respectively. The remaining fin fold was situated ventrally between the pelvic fins and extended posteriorly to the anus. The mouth was terminal throughout metalarval development. The last vestige of median fin fold was absorbed by 29.4 to 31.8 mm, delimiting the end of larval development.

The juvenile period (Figure 2h) was attained at 29.4 to 31.8 mm with final absorption of the median fin fold. The mouth remained terminal on the largest specimen examined (76.3 mm). Juvenile fin ray counts appear in Table 2.

Black buffalo, Ictiobus niger (Rafinesque).--The series consisted of 419 eggs and 502 larvae and juveniles. Morphometric and meristic information (Tables 2 and 4) was recorded from 100 specimens. Mean egg diameter was 2.2 mm (20 eggs) and the range was 2.1 to 2.4 mm. Specimens of cultured young ranged from 5.3 to 25.7 mm.

Protolarval specimens (Figures 3a to 3c) ranged from 5.3 to 8.7 mm. Mean total length at hatching was 5.5 mm (20 specimens, range 5.3 to 5.8 mm). Newly hatched black buffalo were characterized by a decurved head, an unformed mouth, a bulbous, club-shaped yolk sac, and straight notochord. Pectoral buds were present. No other differentiated fins were evident. The dorsal median fin fold originated at the 10th to 12th (11th modally) preanal myomere, was continuous around the urostyle to the anus ventrally, and extended onto the posterior third of the yolk sac.

By 1 day posthatching (6.1 to 6.4 mm) the head was in line with the longitudinal body axis; otoliths were visible as refracting spheres under polarized light; and gill arches were apparent as tissue folds. The mouth was open and subterminal until 7.2 mm, when it became terminal.

By 6.6 to 7.2 mm the gas bladder began to fill. An anterior diverticulum formed from the anterior gas bladder surface by 7.4 to 7.8 mm. Yolk was completely absorbed between 7.2 and 7.5 mm. The tip of the urostyle had flexed and caudal rays formed by 8.8 to 8.9 mm, ending the protolarval phase.

Mesolarval specimens (Figures 3d to 3f) ranged from 8.8 to 17.9 mm. The upper size limit for mesolarvae was not well defined due to the low number of specimens available in larger size classes. Caudal fin rays were first evident between 8.8 and 8.9 mm. By 11.6 mm the urostyle was strongly flexed. An adult complement of caudal fin rays was present by 12.8 to 13.3 mm.

The anterior outline of the dorsal fin became evident as a raised portion of the median fin fold by 8.9 mm. Dorsal fin rays appeared by 12.8 to 13.3 mm and the adult complement was attained by 21.9 mm. Anal fin rays were observed by 15.8 mm.

Pelvic buds were evident as tissue folds along the ventrolateral body wall by 11.5 to 12.6 mm, as wide-based flaps by 13.3 mm, and as narrow-based fins by 17.7 mm. Pelvic fin rays appeared by 17.9 mm. At this size the only remaining fin fold was situated ventrally between the pelvic fins and the anus. Pectoral fin rays appeared by 17.7 mm.

Except on late mesolarvae (15.8 mm or larger) the mouth remained terminal. The upper jaw was even with the lower third of the eye. The gut was straight in specimens as large as 15.8 mm. One full gut coil was present in specimens 17.7 mm or larger. Two distinct gas bladder chambers were present by 13.3 to 14.4 mm.

Only two metalarvae (21.9 and 24.8 mm) were preserved. The adult complement of anal fin rays was present on the 21.9 mm metalarval specimen preserved. Metalarvae (Figure 3g) were characterized by continued pectoral and pelvic fin ray development. The mouth became slightly subterminal and ventral. Metalarvae of the black buffalo retained a fusiform head shape despite the slightly subterminal ventral mouth.

One juvenile specimen (Figure 3h) was preserved (25.7 mm). Fin ray counts appear in Table 2. The head remained smoothly tapered. Squamation was present on both specimens from the caudal peduncle anteriorly along the midline to the area over the gas bladder.

Table 4. Morphometric data ($\bar{x}$ as % of TL or head length with s.d. in parenthesis) for black buffalo.

Total Length Range (Mean Standard Deviation)	N	Percent Total Length: Standard Length	Preanal Length	Predorsal Length	Head Length	Body Depth at Anus	Percent Head Length: Head Depth	Head Width	Snout Length	Eye Diameter
5 3 5 5 (5 4 0 12)	10	96 8 (0 58)	75 5 (0 97)	44 0 (1 94)	15 5 (1 14)	8 5 (0 26)	73 2 (5 14)	63 : (6 32)	23 9 (5 63)	43 7 (2 08)
6 1 6 7 (6 3 0 21)	10	95 9 (0 56)	74 1 (0 93)	36 2 (2 16)	15 4 (0 78)	8 3 (0 44)	75 2 (2 89)	64 8 (2 51)	17 1 (3 76)	40 3 (2 09)
7 1 7 3 (7 2 0 09)	10	94 6 (0 64)	72 5 (2 13)	37 6 (1 76)	16 4 (0 92)	8 5 (0 21)	72 0 (3 43)	64 3 (2 66)	12 6 (2 81)	38 7 (2 74)
8 4 8 9 (8 5 0 21)	8	94 0 (0 72)	72 4 (0 69)	37 4 (1 57)	17 5 (1 67)	8 7 (0 56)	66 0 (2 77)	64 6 (2 67)	13 0 (0 73)	39 9 (1 73)
9 2 9 9 (9 5 0 31)	5	94 4 (1 24)	73 8 (0 87)	44 0 (9 70)	18 4 (0 76)	8 8 (0 51)	64 9 (3 72)	63 3 (4 08)	13 3 (0 57)	38 3 (1 1:)
10 0 10 8 (10 3 0 29)	9	93 3 (1 40)	72 5 (1 47)	38 6 (1 17)	18 3 (0 84)	9 5 (0 e0)	66 8 (2 98)	65 1 (2 31)	13 9 (1 84)	39 8 (1 9:)
11 0 11 7 (11 5 0 25)	10	91 6 (0 95)	71 2 (1 49)	38 5 (1 79)	18 6 (1 23)	9 2 (0 70)	66 4 (4 03)	63 9 (2 99)	15 6 (2 71)	37 9 (2 17)
12 1-12 8 (12 5 0 23)	9	88 2 (0 86)	69 9 (1 67)	38 3 (1 10)	18 6 (0 67)	9 3 (0 36)	67 7 (3 21)	65 1 (2 81)	15 5 (1 58)	38 2 (1 32)
13 0-13 8 (13 4 0 38)	8	87 1 (1 56)	68 3 (2 10)	38 3 (1 48)	19 8 (0 65)	9 6 (0 52)	65 8 (2 82)	62 5 (2 40)	16 3 (1 17)	35 1 (1 40)
14 0 14 9 (14 4 0 33)	9	86 4 (1 23)	69 1 (1 08)	38 2 (1 72)	21 0 (0 85)	9 9 (0 40)	61 4 (3 24)	58 8 (3 35)	16 5 (1 23)	32 5 (2 86)
15 0-15 6 (15 3, 0 20)	7	85 8 (0 73)	69 5 (1 21)	38 7 (1 02)	21 1 (0 63)	10 6 (0 98)	64 2 (2 20)	60 0 (1 41)	18 6 (2 80)	30 4 (2 06)
16 3-16 6 (16 5, 0 25)	2	84 6 (0 54)	67 6 (0 29)	38 1 (0 50)	21 4 (0 32)	10 9 (0 52)	66 5 (2 41)	60 2 (: 61)	19 6 (1 21)	29 3 (2 81)
17 8	1	8e 3	69 1	39 5	22 9	12 3	59 8	57 8	17 2	25 5
24 8	1	78 7	60 6	36 7	23 9	14 6	64 5	51 8	22 4	25 8
25 7	1	79 6	61 2	33 9	24 2	14 6	64 2	57 8	22 5	25 7

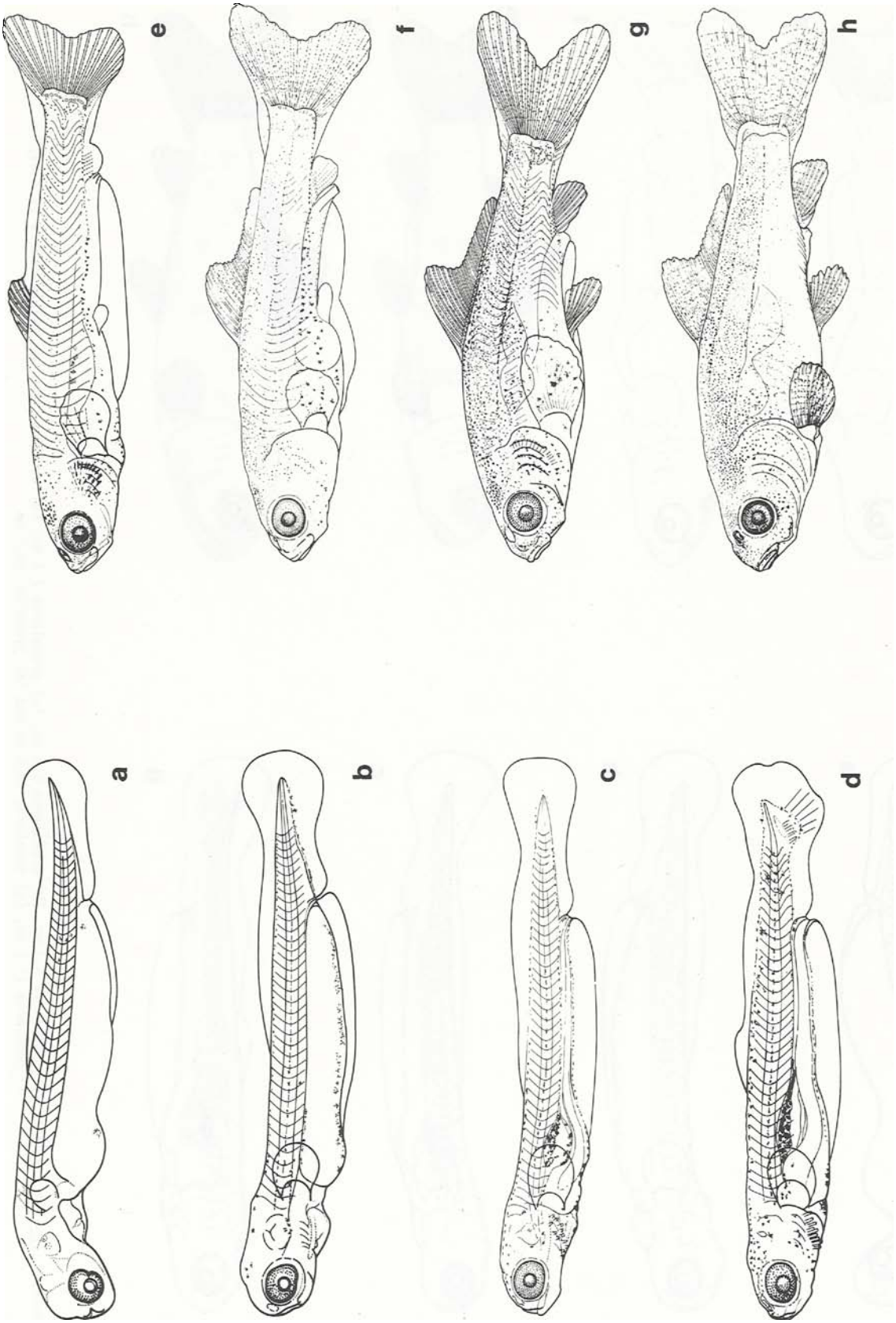

Figure 2. Ictiobus cyprinellus: (a) protolarva 5.6 mm, (b) protolarva 6.5 mm, (c) protolarva 7.2 mm, (d) mesolarva 10.3 mm, (e) mesolarva 16.0 mm, (f) mesolarva 17.4 mm, (g) metalarva 21.3 mm, and (h) juvenile 28.4 mm

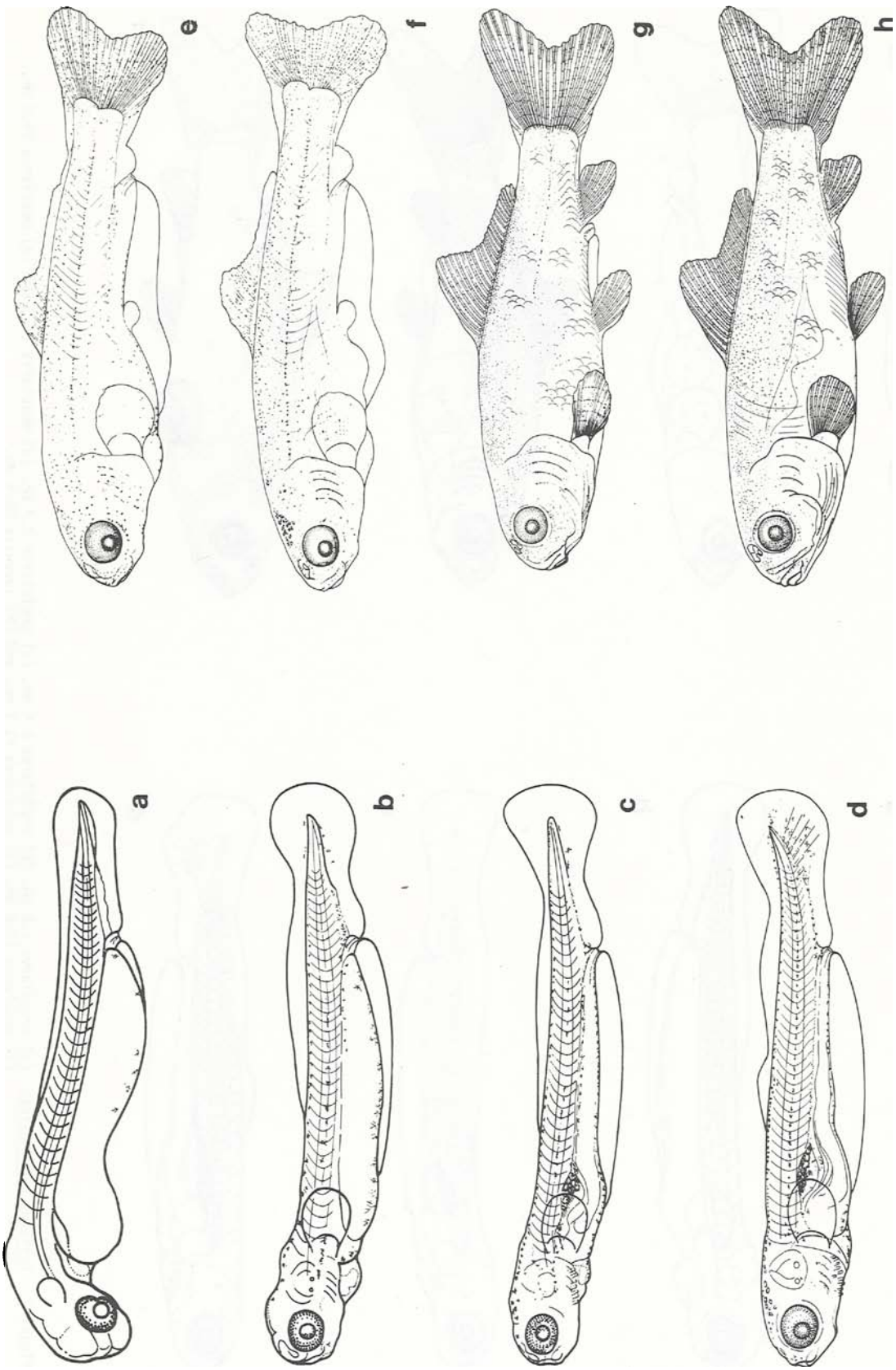

Figure 3. Ictiobus niger: (a) protolarva 5.3 mm, (b) protolarva 6.5 mm, (c) protolarva 7.4 mm, (d) mesolarva 9.0 mm, (e) mesolarva 15.2 mm, (f) mesolarva 17.1 mm, (g) metalarva 24.8 mm and (h) juvenile 25.7 mm

DISCUSSION

A combination of characters, including number of preanal myomeres, size at hatching, size at particular stages of development, and pigmentation patterns, is diagnostic for the subfamily Ictiobinae and the genus Ictiobus. The cumulative range of preanal myomeres for all species of Ictiobinae found in the United States is 26 to 31 (very rarely 32). Only the ranges for Erimyzon oblongus 30-33 (Fuiman 1979a), E. sucetta 27-29 (Fuiman 1979b), Minytrema melanops 31-35 (Hogue and Buchanan 1977), Moxostoma erythrurum 31 to 37 (Fuiman and Whitman 1979), and M. macrolepidotum 30-35 (Buynak and Mohr 1979) overlap those of the Ictiobinae.

Larvae of Erimyzon may be separated from those of Ictiobus on the characteristic lack of pigment on the occiput in Erimyzon (Fuiman 1979a) and the comparatively different sizes at particular states of development. Gross body morphology and pigmentation patterns (Hogue and Buchanan 1977) serve to adequately separate larvae of M. melanops, which hatch at a size similar to that of ictiobines and occasionally have 31 to 32 preanal myomeres. Moxostoma spp. and Hypentilium nigricans (Fuiman 1979a, Fuiman and Whitman 1979, Yeager 1980) hatch at larger sizes than ictiobines and attain similar stages of development at much larger sizes. Once differentiation of the dorsal fin fold has begun, the long dorsal fin base and associated greater number of dorsal fin rays (Table 2) is diagnostic for the ictiobines.

Table 5. Total length (mm) when morphological changes appear during ontogeny of three buffalos.

	I. bubalus	I. cyprinellus	I. niger
Mean (range) hatching size	6 8 (5 0 to 6 3)	5 6 (5 0 to 6 3)	5 5 (5 3 to 5 8)
Presence of 1st gas bladder chamber	6 4 > 8	6 5 9 8	6 6 7 2
2d gas bladder chamber	12 8 14 2	14 2 16 5	13 3 14 4
Yolk sac absorbed	6 9 7 5	6 7 7 3	7 2 7 5
Gut coiling begins	16 5 18 0	15 5 16 2	15 8 17 7
Full coil present	16 0	16 9	17 7
Protolarval Phase	5 0 9 5	5 0 9 4	5 3 8 7
Mesolarval Phase	9 5 23 1	9 4 21 3	8 8 = 17 9
Metalarval Phase	22 2 30 5	22 1 31 8	21 9 > 24 8
Juvenile Phase	27 5 ?	29 4 ?	25 0 ?
Beginning/Completion of fin ray development			
Caudal	9 6/12 6 14 2	9 4 10 0/13 0	8 8 8 3/12 8-13 3
Dorsal	15 1/22 2 23 6	13 9/21 0 23 5	12 8-13 3/21 9 (=)
Anal	15 2 18 0/21 0 23 6	15 3/20 3	15 8/21 8 (=)
Pectoral	17 8 18 0/27 5 30 6	15 3 16 5/22 1 24 3	17 7/24 8 (=)
Pelvic	17 8 18 0/23 6 26 1	15 3 16 5/22 1 23 5	17 5/24 8 (=)
Pelvic buds present	12 6	11 5	11 5/12 6

Overlap of preanal and total myomere counts for Ictiobus and Carpiodes is so complete (Yeager 1980) as to preclude independent diagnostic value for separating the two genera. However, the more elliptical eye and flattening of the head, characteristic of the carpsuckers, allow ease of identification of the genera at sizes greater than 8 mm. Typically the midventral line of melanophores on early protolarval carpsuckers (< 8 mm) is more diffuse than that of the buffalofishes.

Despite acquisition of extensive meristic and morphometric data, observations on gross morphology, sizes at particular stages of development (Table 5), and pigmentation patterns, no single character or combination of characters for specific identification of early buffalo fish larvae was obtained. Larval smallmouth and black buffalos >16 mm have slightly subterminal mouths and distinctly subterminal mouths by 20 mm, as opposed to the condition in bigmouth buffalo, which retains a terminal mouth throughout larval development.

The vagueness of distinguishing characteristics of larval fishes in the genus Ictiobus is not surprising. Hubbs et al. (1943) in dealing with adult buffalofishes stated that the species are "characterized by a combination of traits, none of which by itself may be invariably distinctive." Intraspecific variations in larvae were so great as to mask differences between species. Characters commonly employed at higher taxonomic levels in larval fish identification, such as myomere counts, pigmentation patterns, morphometrics, and gross body morphology, were not definitive at the species level.

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COMPARATIVE DEVELOPMENT OF REDFIN PICKEREL (Esox americanus americanus) AND THE EASTERN MUDMINNOW (Umbra pygmaea)

by

Robert Malloy and F. Douglas Martin

Egg stages and yolk-sac larvae of the redfin pickerel (Esox americanus americanus) and the eastern mudminnow (Umbra pygmaea) are poorly known despite the abundance of information on congeners of both. Both of these species were reared in the laboratory from artificially fertilized eggs. Developmental sequences of both species are very similar down to and including previously unreported patterns of movement of the oil droplets. Newly hatched yolk-sac larvae are similar in appearance but are easily distinguished by size, color pattern, and the number of myomeres between the yolk sac and the anus.

INTRODUCTION

The redfin pickerel (Esox americanus americanus) and eastern mudminnow (Umbra pygmaea) are found in the tidal tributaries of the Chesapeake Bay. These fishes are common, but their early life history stages are poorly described (Jones, Martin and Hardy 1978). We find them to be similar to each other but easily distinguishable, and are presenting some previously unreported information which may have systematic importance.

Both species spawn in the early spring. U. pygmaea deposits eggs at the base of shoreline vegetation or in algal mats (Jones, Martin and Hardy 1978), and E. a. americanus disperses eggs over flood plains (Crossman 1962). Although egg development appears to be typical of teleostean embryogenesis, we found a unique pattern of movement of oil globules common to both species. Larval development is similar to other members of the suborder Esocoidei (Klienert and Mraz, 1966; Jones, Martin and Hardy, 1978; and others) and distinguishing characteristics are discussed.

METHODS AND MATERIALS

Adults of both species were collected from Helens Creek, a tributary of the Patuxent River, in Calvert County, Maryland, using unbaited minnow traps. At the time of collection, the water temperature was 3.5 C. Adults were transported to the Chesapeake Biological Laboratory where eggs from ripe females of each species were stripped into watch glasses and fertilized by adding the milt from a macerated testes of an appropriate male. Eggs were incubated in a refrigerator at 1 to 6 C. Although this temperature is below recorded incubation temperatures (Jones, Martin and Hardy 1978; Wang and Kernehan, 1979), it was chosen because gravid adults were captured at these temperatures, and because attempts to rear these species in previous years had indicated mortality related to temperature of 15 C. Initial observations were made hourly for 24 hours. After that eggs were observed at 2- to 8-hour intervals until hatching. Larval development was controlled at 10 to 12 C and observed twice daily.

RESULTS

Egg development for both species was apparently typical of teleostean embryogenisis with some notable peculiarities, one of which was the movement of the oil globules. Oil globules congregated under the developing morula, then dispersed with somite formation. This movement changed the optical density of the yolk and wakes were left by the moving oil globules. These oil globules later reaggregated in the larvae (Figure 1).

The embryonic heart was S-shaped, forming anterior and to the left of the head. Heart beat began within 4 days of fertilization.

Hatching was noticeably different between the two species in our study. U. pygmaea hatched yolk first through a small slit in the chorion contorting the head and body (Figure 2). This may be an artifact of laboratory conditions, but was consistent, and may have contributed to an 80% hatching mortality rate. E. a. americanus hatch head first, seemingly putting little stress on the hatching embryo (Figure 3). This species had an 80% hatching success.

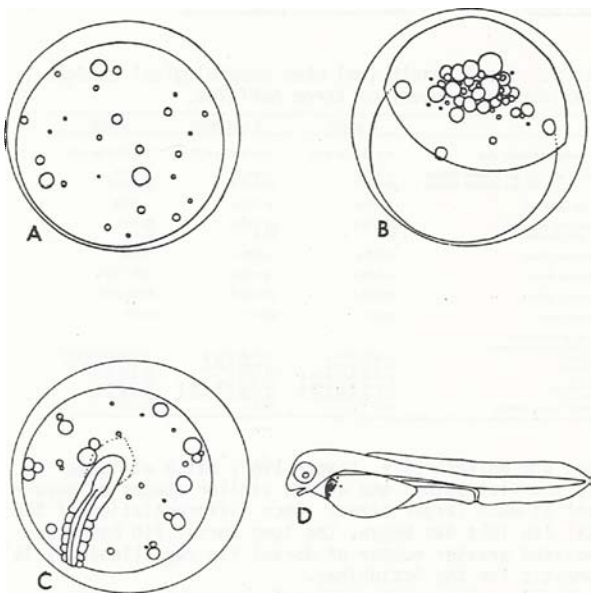

Figure 1. A diagrammatic representation of oil droplet movements. A. A recently laid egg with dispersed oil droplets. B. Morula stage with clustered oil droplets. C. Later embryo with redispersed oil droplets. D. Larva with reaggregated oil droplets.

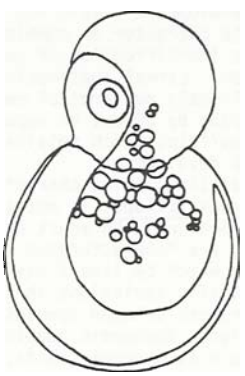

Figure 2. Umbra pygmaea hatching by forcing a bleb of yolk material through an opening in the chorion.

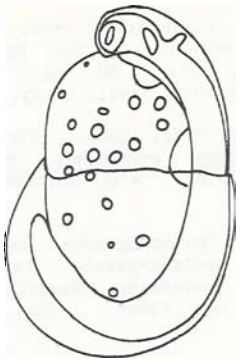

Figure 3. Esox a. americanus hatching headfirst.

Yolk-sac E. a. americanus hatched at 7.2 mm total length; the head was deflexed and attached to the yolk sac and the mouth was not formed. The head, thorax and dorsal third of the yolk sac were pigmented with small stellate melanophores, and a distinctive single dark stellate melanophore was on the anus. The heart was on the left side of the head, just below the otic capsule during this stage. The vitelline circulation system consisted of a single hepatic vein (on the left of the yolk sac) which joined the subintestinal rete that covers the posterior third of the yolk sac. The subintestinal vein was joined by symmetrical common cardinal veins as it entered the sinus venosus (Figure 4.).

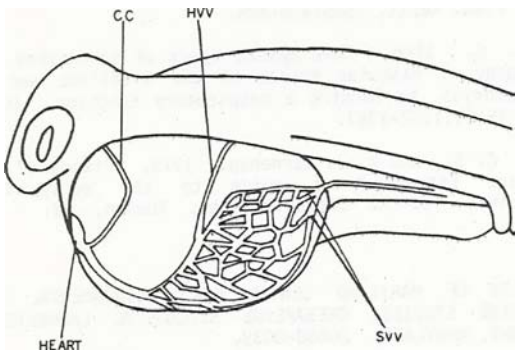

Figure 4. Newly hatched E. a. americanus showing the vitelline circulatory pattern.

U. pygmaea hatched at 5.4 mm total length with no visible mouth parts and the head deflexed and attached to the yolk sac. There was very little pigmentation except for a few small melanophores on the top of the head and a large stellate melanophore on the anus. As in Esox the heart was on the left side of the head below the otic capsule. The hepatic vitelline vein was paired and symmetrical, branched in three places before entering the subintestinal rete which covered the posterior two thirds of the yolk sac. The common cardinal veins descended across the anterior fifth of the yolk sac and joined the subintestinal rete at the sinus venosus (Figure 5).

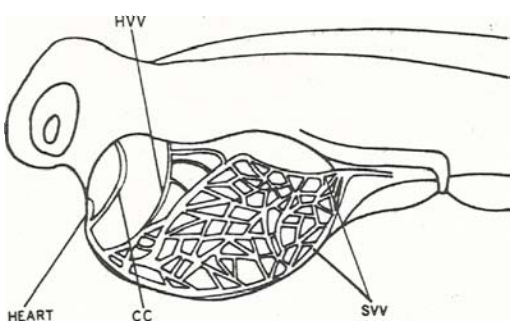

Figure 5. Newly hatched U. pygmaea showing the vitelline circulatory pattern.

After one week E. a. americanus yolk-sac larvae have a dark pigment streak from the eye to the hypural anlagen. The dorsal finfold was pigmented, as are the eyes and branchial arches, and the anal melanophore was distinctive. The heart was folded into the body cavity. The common cardinals and hepatic vein also migrated into the body cavity, and the subintestinal was the only vein on the ventral portion of the yolk sac.

After one week yolk-sac Umbra larvae were heavily pigmented on the head and on the dorsal part of the body. The eyes were also heavily pigmented. Meckel's cartilage was formed, but the head was still partially attached to the yolk sac. Also, the heart was rotated into the body cavity as in Esox.

DISCUSSION

The similarities of the embryonic heart and vitelline venous system support the hypothesis of phylogenetic grouping of Esocidae and Umbridae. The formation of the heart anterior to and on the left side of the head is unusual. There are two more common positions. The most common is within the pericardial cavity as displayed by Gobio gobio (Penaz and Prokes 1978), Percina spp. and Etheostoma spp. (Martin, pers. obsv.), Microgadus tomcod (Hardy 1978a; Hardy and Hudson, 1975), and Rhodeus o. ocellatus (Nagata and Hyabe 1978). The other common placement is in front of the head as found in Tylosurus crocodylus, Fundulus diaphanus, F. heteroclitis, F. majalis, Lucania parva and Gambusia affinis (Hardy 1978b). Illustrations of cyprinodonts show that the heart may sometimes be displaced either left or right, but never far from the midline as noted for our specimens.

Yolk-sac circulatory patterns have been offered as potential tools for determining phylogenetic relationships (Soin 1966, Martin and Hubbs 1973). Martin and Hubbs (1973) offered a system of classifying those circulatory patterns. Their system was naive and would have benefitted from an examination of Soin (1966) and Kunz (1964). Their basic statements about Esox were based on Ryder (1887) and the pattern illustrated by Ryder differs from the pattern seen in our Esox larvae. Martin and Hubbs state that for Esox there are four main vessels entering the sinus venosus. Ryder's illustration shows what might be interpreted as paired sections of the subintestinal vitelline vein (SVV) plus multiple branches, that may be part of the hepatic vitelline veins (HVV). Our own observations show that the SVV is highly branched and interconnected but not paired and common cardinal veins are present (not indicated by Ryder).

Soin (1966) does not state which species of Esox he examined, but he does not show any branches of the HVV. We do not know if this difference between his description and ours is due to error in one publication or the other or if the differences are real, however we suspect that the illustration by Soin is based on a larva where the heart is moving off the yolk sac into the permanent position in the pericardial sac. If this is the case, the HVV branches may have already migrated off the yolk and the patterns are consistent between the two studies.

Movement of the oil globules during embryogenesis was reported in the Bathylagidae, a Salmoniformes family. Ahlstrom (1968) reported similar oil globule migration in Bathylagus and Lueroglossus. The oil globules in these species exhibit the same initial migration under the morula as exhibited by Umbra and Esox.

Identification and separation of U. pygmaea and E. a. americanus yolk-sac larvae is accomplished through three characteristics. Esox is more heavily pigmented, especially the stripe along the side of the body. Although the body shape is the same, Esox is larger. Finally, the myomere count from yolk-sac to anus in Esox is 12 and in Umbra is 5. Variation for this last character is not adequately known, considering the variability in vertebra number shown in Esox species (Crossman and Buss 1965).

In conclusion the similarity of embryogenesis and larval circulation pattern indicate a phylogenetic relationship within the currently defined suborder Esocoidei. The pattern of movement of the oil droplets suggests a possible relationship to the Bathylagidae; however, not enough is known about oil droplet movements in other groups of fish to state that this oil droplet movement pattern is not a more widespread phenomenon.

ACKNOWLEDGMENTS

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DEVELOPMENT OF LARVAL POLYODON SPATHULA (WALBAUM) FROM THE CUMBERLAND RIVER IN TENNESSEE

Bruce Yeager and Robert Wallus

ABSTRACT

Adult paddlefish were collected from the Cumberland River in Tennessee and induced to spawn with pituitary injections. Eggs were cultured under hatchery conditions in McDonald jars at 14 to 19 C and the larvae transferred to aquaria and then concrete raceways at 20-24 C. Eggs hatched from 155 to 166 hours after fertilization. Average egg diameter was 3.5 mm. At hatching, larvae were 8.1 to 9.0 mm total length (TL). Paddlefish have 32-35 preanal and 22-26 postanal myomeres respectively. Eyes remained small throughout development. Tooth buds appeared on the jaws, tongue, and palate by 13 mm TL (2½ days posthatching). Sensory patches were present by 15 mm TL. All fins except the caudal had adult ray counts by 89 mm TL. The preanal myomere count, relative eye size, and more anterior origin of the dorsal finfold on the paddlefish allow taxonomic separation from all other superficially similar larval. Cannibalism occurred among larvae between 18 and 87 mm TL.

INTRODUCTION

In spite of extensive life history work (Adams 1942; Robinson 1966; Friberg 1972; Pasch et al. 1978) on Polyodon spathula (Walbaum) information concerning morphological development of this species is limited. Wagner (1904), Nachtrieb (1910), Allis (1920), and Larimore (1949) studied the anatomy of adult paddlefish, and Ballard and Needham (1964) provided an excellent study of embryonic stages and larvae through the onset of feeding at about 15 mm TL, approximately 10 days after hatching. Early accounts by Allen (1911), Barbour (1911), Danforth (1911), and Thompson (1933) described a few young paddlefish ranging in total length from 17 to 300 mm. Purkett (1961) described seven newly hatched paddlefish larvae (8.0-9.5 mm TL) and one 29-day-old specimen (47.9 mm TL).

The objective of this investigation was to provide a continuous description of the morphological development of Polyodon spathula from hatching to the juvenile period. This description will complement previously published work and provide information on many as yet undescribed stages and assist other investigators in distinguishing paddlefish larvae from similar larvae of acipenserids, lepisosteids, and hiodontids.

METHODS

Between April 19 and April 21, 1978, paddlefish in near spawning condition were captured in 12.7-cm bar-mesh gill nets fished at night in the Cumberland River below Cordell Hull Dam. The morning after capture, broodstock were given interperitoneal injections of macerated paddlefish pituitary. Female paddlefish first released eggs 28 hours after injection of pituitary material. Eggs began flowing freely (about 10,000-15,000 per stripping) 34 hours after injection. Multiple spawns were obtained with each female stripped about every hour. Eggs were placed in McDonald jars in lots of approximately 100,000. River water at 15 C was used to incubate the eggs for 12 to 16 hours until they were shipped to Cohutta National Fish Hatchery, Cohutta, Georgia, where they were incubated at temperatures ranging from 14.4 to 18.8 C.

Eggs were shipped from Cohutta to Tennessee Valley Authority (TVA) laboratory facilities in Norris, Tennessee, and hatched soon after arrival. Larvae were placed in 114 l aquaria and held for 10 days in aerated spring water and then transferred to a 2.23 m x 6.10 m x 9.91 m concrete raceway supplied with a low volume of spring water. Temperatures ranged from 19.5 to 21.0 C in the aquaria and from 20.5 to 24.0 C in the raceway. An initial food culture, begun with 25 pounds of sheep manure, a bale of hay, 3 ounces of brewer's yeast, and wild zooplankton, was previously established in the raceway. Additional feeding with brine shrimp and zooplankton supplemented the food supply. Developing paddlefish were preserved daily in cold 10 percent Formalin and later transferred to buffered 5 percent Formalin.

Meristic and morphometric data were obtained using a stereomicroscope equipped with an ocular micrometer. Characters examined were total, snout, preanal and postanal lengths, and counts of preanal myomeres, postanal myomeres, fin pterygiophores, and rays. Methods of obtaining lengths and counts were as described by Hogue et al. (1976). Head development and pigmentation patterns were particularly noted. Selected morphometric data were analyzed for allometry from log-log plots. Regression equations were calculated using Bartlett's (1949) method for both variables measured with error. The equations are of the form $\log Y = \log b + \alpha \log X$, the logarithmic form of Huxley's (1932) equation for growth, $Y = bX^{\alpha}$, where Y is the size of one body part, X the size of the other body part and b and α are constants. In the log transformed form, α is the slope of the log-log line and b the index of the size of part Y when X is of unit size. Multiple growth stanzas (Martin 1949, Fuiman and Corazza 1979) were used to exemplify rate changes.

The specimens examined in this study are part of a series of 159 eggs and 2,857 Polyodon larvae, supplemented with juvenile specimens collected from TVA power plant intake screens. All specimens in this series are cataloged as TV909, DS-21 in the reference collection of the Larval Fish Identification and Information Center, Tennessee Valley Authority, Norris, Tennessee.

RESULTS

Eggs and Hatching.--Early embryonic paddlefish eggs were gray, demersal and very adhesive. Except for the grayish yolk material, embryos near hatching had little pigment. Ventrally, a small dark bar of pigment was observed immediately anterior to the tip of the notochord, and laterally, diffuse bands of melanophores were sometimes present from the nape to the vent.

Immediately prior to hatching, 10 eggs averaged 3.5 mm and ranged from 3.3 to 3.9 mm in diameter. The eggs hatched from 155 to 166 hours after fertilization. Prehatching mortality for the cohort used in this study was approximately 10 percent.

Embryos emerged tail first, becoming free of the egg capsule by intense writhing as described by Purkett (1961). Newly hatched larvae averaged 8.4 mm TL (20 specimens, range 8.1 to 9.0 mm TL).

Internal Organogenesis.--A considerable amount of dark gray yolk was present at hatching (Fig. 1a). The post gut was present but empty at this stage. Ballard and Needham (1964) indicated yolk absorption was complete by 15 mm TL (approximately 10 days post hatching). Our specimens retained yolk material until 17 mm TL, approximately 6 days after hatching. Ballard and Needham's (1964) detailed description of internal organ development was in accord with that of specimens examined in this study.

Head Development.--At hatching the head was strongly decurved over the yolk sac. Barbel buds were apparent on many larvae. Otic placodes were present but otoliths were absent. Opercular flaps were short, not covering the incipient gill folds. The nasal openings were single and rounded and the stomodeum if present was not well developed. Eyes were present but small, which is characteristic of this species throughout its development.

By 10 to 11 mm TL (1½ days after hatching) the head was in line with the body axis. Barbel buds were present beneath the eyes on all larvae, and the mouth was open. Gill filaments were approximately 0.2 mm in length and were covered by the operculum. By 13 mm TL the gill filaments extended beyond the posterior edge of the opercle. Eyes remained small and pigmentation was present in the optic cups. The nasal opening had become oval as ingrowing tissue began dividing the aperture. Sensory patches were profuse on the operculum (Fig. 1b).

Tooth buds appeard on the jaws, tongue, and palate by 13 mm TL (2½ days post hatching). By 17 mm TL the teeth were strongly recurved.

By 15 mm TL anterior and posterior nasal apertures divided by fusion of ingrowing tissue. Cartilage in front of the brain had begun to extend anteriorly, giving the snout a rounded appearance. Sensory patches covered the opercle and head with the heaviest concentration under the eye and ventrally on the snout anterior to the barbels.

At 17 mm TL (Fig. 1c) the barbels were longer than wide and located in front of the eye even with the anterior edge of the nasal opening. The snout was about 1 mm long. The eyes and nasal openings shifted forward relative to the brain. The opercle once again covered the gills and had lengthened posterio-dorsally beyond the pectoral fin. Auditory pits were visible posterior to the eyes. Sensory patches covered the dorsal and lateral portions of the head, opercle, snout, and rostrum (Fig. 1d). The only portion of the head lacking these sensory rosettes was immediately ventral to the mouth.

At total lengths greater than 24 mm the ventral profile of the laterally viewed head was smoothly tapered; the forward extension of cartilage in the developing snout streamlined the upper jaw. Rostral development on specimens greater than 89 mm TL was characterized by continued growth in length and thinning of the basal width to the adult spathulate form.

Fin Development.--On newly hatched larvae (8.1 to 9.0 mm TL), the median finfold began dorsally at the 16th or 17th preanal myomere and was continuous around the urostyle to the vent and from the vent anteriorly to the yolk sac (Fig. 1a). Dorsally, the emarginate nature of the finfold indicates the future position of the dorsal fin (Table 1).

Caudal fin differentiation began by 10 mm TL as an opaque area of the finfold. By 13 mm TL the caudal finfold had developed a long, deep ventral margin, and the posterior portion of the notochord was located much closer to the dorsal margin of the caudal finfold. On most specimens the caudal finfold had attained the heterocercal form characteristic of the adult caudal fin by 17 mm TL (Fig. 1c). By 34 mm TL a distinct notch had developed ventrally near the tip of the caudal fin, giving the distal edge a lobed appearance (Fig. 1e). The fin posterior to the notch had no basal elements or fin rays until about 145 mm TL.

The adult complement of fin rays was present in each fin long before rays were developed to the fin margin (Fig. 1f). The fins attained adult ray counts in the following sequence: dorsal, anal, pelvic, pectoral, and caudal, with adult complements present in all but the caudal fin by 89 mm (Table 1, Fig. 1g). Segmentation of fin rays was apparent soon after individual rays were discernible. The development of all fins was completed between 145 and 160 mm.

Table 1. Fin development of young paddlefish (total lengths in millimeters).

Fin	Dorsal	Anal	Pelvic	Pectoral	Caudal
Fin outline differentiation begins	9	11-13	11-12	9-10	12
Countable fin rays present	26	28	35-36	35	28
Adult complement of fin rays present	65-70	65-70	79-90	89	145
Completion of fin ray development	145	145	145-160	145-160	145-160

Pigmentation.--Within 12 hours after hatching the eyes became pigmented, and four or five scattered melanophores formed dorsal to the tip of the notochord. The dark bar ventral to the tip of the notochord disappeared by 13 mm TL.

Between 10 and 11 mm TL (1½ days post hatching) the area of the midbrain became pigmented laterally and the hindbrain dorsally. Dense pigmentation was present both laterally and dorsally along the brain by 15 mm TL. Dorsally, the forebrain was "stitched" with melanophores around the edge. A distinct middorsal line of melanophores divided the forebrain. The head became more densely pigmented dorsally with increasing size.

By 13 mm TL there was internal pigment on the surface of the notochord anterior to the anus (Fig. 1b) and to the urostyle by 15 mm TL. Melanophores were also present down the myoseptae to about the midline. From 15 mm to 24 mm TL, external lateral pigmentation was scattered but became progressively heavier beginning dorsally. Lateral pigmentation along the opercle behind the eye was first apparent at about 24 mm TL; and the snout, ventrum of the forebrain, gill arches, and dorsum of the gut were also pigmented. Midlateral pigment was in blotches along the flanks down to the midline by 24 mm TL, and larvae were only lightly pigmented ventrally. Pigmentation did not change significantly beyond this length. Internal organs were still visible on specimens as large as 135 mm. The rostrum was pigmented from the onset of development.

Behavior.--At hatching paddlefish larvae were capable swimmers, traveling randomly for long distances without pause in activity. As noted by Purkett (1961), 2- to 3-day-old larvae swam incessantly. Sudden changes in direction were common. Larvae of about 15 mm TL were frequently observed swimming with their mouths open wide. Larvae smaller than 25 mm TL tended to stay near the water surface. At larger sizes they remained deeper. A dense phytoplankton growth in the tank hindered observation, but few larvae were noted near the surface. Except in the early morning, larvae of all lengths were seldom observed near the surface.

Active feeding began when larvae had absorbed the yolk (between 16 and 17 mm TL). The larvae readily ingested whatever food was offered, e.g., live brine shrimp, frozen brine shrimp, pulverized trout chow, wild

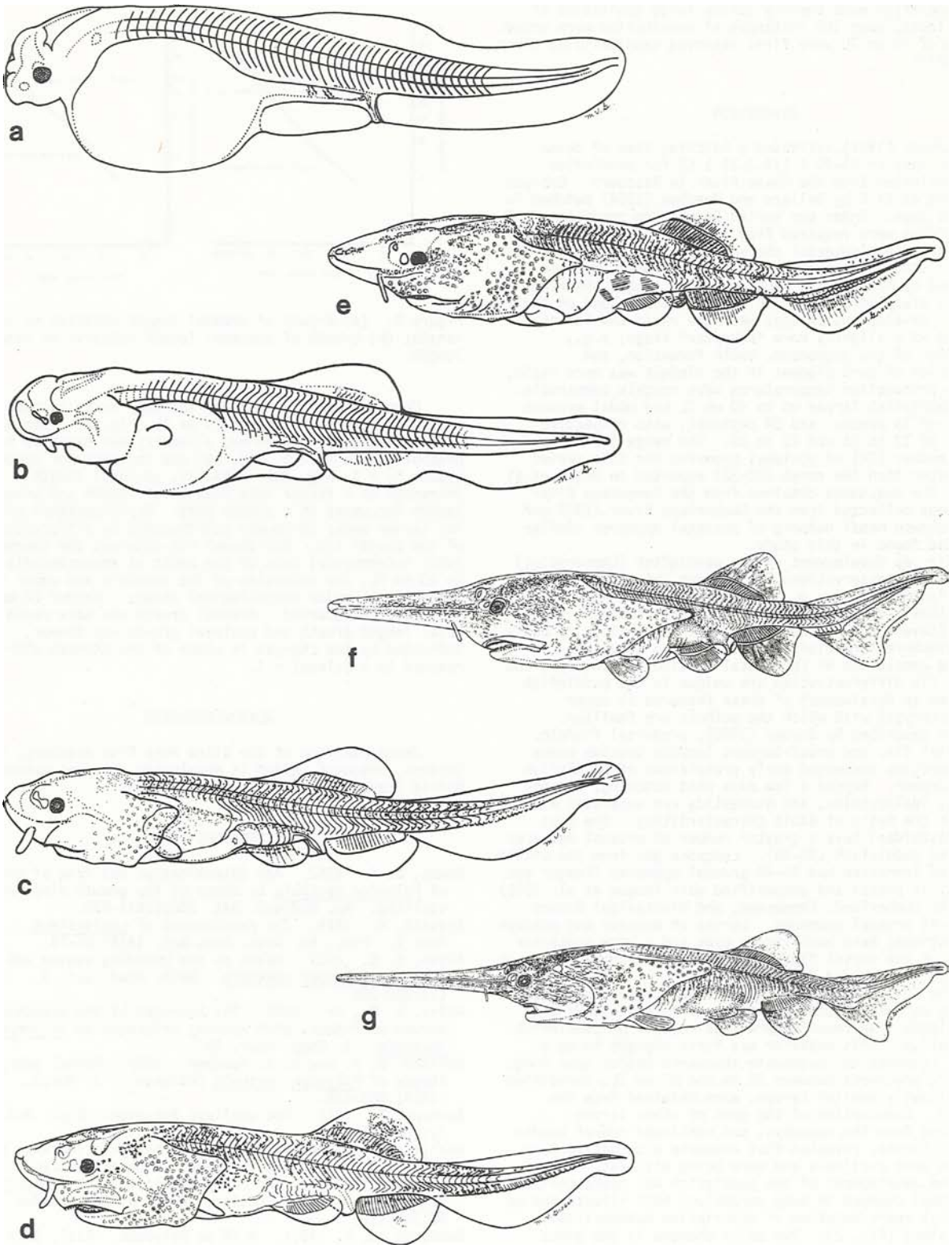

Figure 1. Larvae of Polyodon spathula: (a) 8.9 mm newly hatched; (b) 13 mm; (c) 17 mm; (d) 21 mm; (e) 34 mm; (f) 54 mm; (g) 89 mm.

zooplankton (mostly Daphnia pulex), and striped bass (morone saxatilis) larvae. The guts of preserved specimens contained all these foods in quantity. Although the paddlefish were readily taking large quantities of other foods, over 100 instances of cannibalism were noted. Larvae of 18 mm TL were first observed cannibalizing other paddlefish.

DISCUSSION

Purkett (1961) estimated a hatching time of seven days or less at 65-70 F (18.3-21.1 C) for paddlefish eggs collected from the Osage River in Missouri. Embryos cultured at 14 C by Ballard and Needham (1964) hatched in 9 to 10 days. Under our varied incubation conditions 6.5 to 7.0 days were required from fertilization to hatching.

Early developmental characteristics of the paddlefish in this study corresponded for the most part to those observed by Ballard and Needham (1964). Eggs observed in this study were larger in diameter than those of their study. Development of eggs was more rapid and larvae hatched at a slightly more "advanced" stage; e.g., formation of the stomodeum, tooth formation, and collection of dark pigment in the hindgut was more rapid, though propagation temperatures were roughly comparable.

Paddlefish larvae up to 40 mm TL had modal myomere counts of 33 preanal and 24 postanal, with respective ranges of 32 to 35 and 22 to 26. The range (22-26) and modal number (24) of postanal myomeres for this series is greater than the range (20-22) reported in Hogue et al. (1976) for specimens obtained from the Tennessee River. Specimens collected from the Cumberland River (1977 and 1978) showed modal numbers of postanal myomeres similar to those found in this study.

Fin ray development of the paddlefish (Chondrostei) differs from observations for various Teleostei, or for other "primitives," e.g., gars (Wilder 1833, Agassiz 1879, Beard 1889), the bowfin (Dean 1896), or sturgeons (Ryder 1890, Stevens and Miller 1970). The combination of early pterygiophore formation, delayed fin ray formation, delayed completion of the distal portion of fin rays, and caudal fin differentiation are unique to the paddlefish compared to development of these features in other Actinopterygii with which the authors are familiar.

As described by Snyder (1980), predorsal finfold, predorsal fin, and snout-to-vent lengths provide means of separating undamaged early protolarvae of paddlefish and sturgeon. Beyond a few days post hatching, acipenserids, lepisosteids, and hiodontids are separated with ease on the basis of adult characteristics. The gars (Lepisosteidae) have a greater number of preanal myomeres than the paddlefish (32-35). Longnose gar from the Little River in Tennessee had 43-46 preanal myomeres (Yeager and Bryant, in press) and unspecified gars (Hogue et al. 1976) from the Cumberland, Tennessee, and Mississippi Rivers had 39-44 preanal myomeres. Larvae of mooneye and goldeye (Hiodontidae) have much larger eyes and a more posterior origin of the dorsal finfold than the paddlefish (Hogue et al. 1976, Snyder and Douglas 1978).

The only reported instances known to us of paddlefish feeding on other fishes are those of Forbes (1888) and Fitz (1966). We found no previous reports of paddlefish cannibalism. This behavior was first thought to be a stress response to inadequate abundance and/or type food. However, specimens between 35 mm and 87 mm TL, containing only slightly smaller larvae, were obtained from the raceway. Examination of the guts of other larvae collected from the raceways, and continued robust condition of larvae, revealed that adequate alternative food sources were available and were being utilized.

The development of the paddlefish was characterized by gradual changes in body morphology best illustrated as graphical representation of descriptive mathematical expressions (Fig. 2). Two major changes in the gross morphology of the paddlefish (i.e., caudal fin and rostral development) were manifested along a linear body axis and were uncomplicated enough to be sufficiently specified by the log form of the power regression or simple "allometry" expression ($Y = bx^{\alpha}$). Isometric growth (two body parts growing at the same rate) would be indicated by the slope of the log-log transformed regression, $\alpha = 1$. Allometric ($\alpha \neq 1$) rather than isometric growth for preanal and postanal development is indicated (Fig. 2) for the paddlefish.

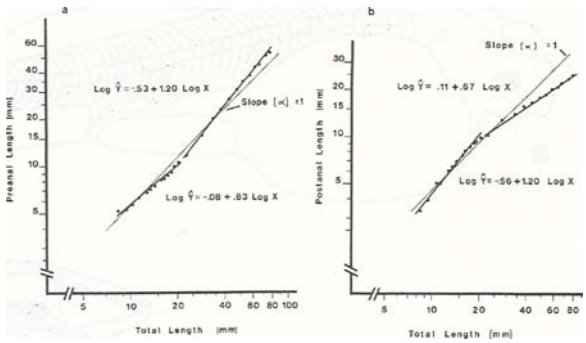

Figure 2. (a) Growth of preanal length relative to total length; (b) growth of postanal length relative to total length.

Change in growth rate for the body parts in paddlefish became discernible at about 20 mm TL (Fig. 2). For paddlefish larvae less than 20 mm, allometry was negative for preanal growth ($\alpha < 1$, Fig. 2a) and positive for postanal growth ($\alpha > 1$, Fig. 2b). That is, postanal length increased at a faster rate than total length and preanal length increased at a slower rate. Rapid postanal growth for larvae under 20 mm was attributable to differentiation of the caudal fin. The caudal fin attained the characteristic heterocercal form of the adult at approximately 17 to 20 mm TL; the extension of the urostyle and upper find lobe was the major morphological change. Beyond 20 mm TL the converse occurred. Preanal growth was more rapid than total length growth and postanal growth was slower, indicated by the changes in slope of the stanzas with respect to α (slope) = 1.

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VENTRAL PIGMENT PATTERNS OF *Alosa aestivalis* AND *A. pseudoharengus* LARVAE

Karen Ripple, Phillip Jones, and F. Douglas Martin

ABSTRACT

Ventral pigment patterns of larval *Alosa aestivalis* and *A. pseudoharengus* exhibit such overlap and variation that they can not be used as reliable criteria for distinguishing these congeners. Three basic patterns in the former and five patterns in the latter species were witnessed in subsamples from Potomac River collections. However, no pattern was seen in more than 65% of specimens of either species.

INTRODUCTION

Ichthyoplankton from the Potomac River were examined in search of a rapid means of sorting large numbers of *Alosa* species larvae taken together when sampling estuarine portions of the river. Four *Alosa* species occur in the Potomac estuary (Hildebrand and Schroeder 1928). Larvae of *A. aestivalis* (Mitchill) and *A. pseudoharengus* (Wilson) have been abundant in ichthyoplankton collections of recent years, while larvae of *A. mediocris* (Mitchill) and *A. sapidissima* (Wilson) have been seen considerably less often and may now be absent from the estuary (personal observation). *Alosa* species spawn at or above the freshwater/saltwater interface of the Chesapeake Bay and its tributaries throughout the spring and early summer. In addition, *Dorosoma cepedianum* (Lesueur) and *D. petenense* (Gunther) larvae are collected in freshwater in early summer (Jones et al. 1978).

Methods of identifying these larvae are time consuming, relying primarily on the location and date of collection, fin ray and myomere counts (Lippson and Moran 1974, Chambers et al. 1976), and pigment patterns (Leim 1924, Wang and Kernehan 1979). Fin rays are undeveloped on most larvae sorted. In addition, myomere counts are possible only when the larvae are undamaged by the net. If pigment patterns could be used along with collection date and location to sort these species, handling time would be much reduced.

METHODS

Larvae were randomly subsampled from 1977 and 1981 ichthyoplankton collections from the Potomac River between Stuart Point and Hallowing Point. All larval sizes were not observed due to termination of ichthyoplankton sampling in early June. Pigment patterns of 26 *A. aestivalis* and 94 *A. pseudoharengus* larvae were examined and compared. Dorsal and anal fin ray counts (where fins were sufficiently developed), preanal myomere counts, and dorsal fin to vent myomere counts were made to assure positive identification.

RESULTS

The most typical ventral pigmentation pattern for *A. aestivalis* was observed on 16 larvae (62%) and begins with 1-3 melanophores along the mid-line of the isthmus between the gills, then 206 melanophores in parallel rows posterior to the cleithrum, a short break, then parallel rows resuming along the junction of the body and the intestine (Fig. 1a). Only 2 larvae (7%) exhibited the pattern described by Leim (1924) in which the melanophores diverge into two straight rows forming a V-shaped pattern between the cleithrum and the future site of the pelvic fins (Fig. 1b). A third pattern (Fig. 1c) in which the diverging rows curve was observed on 6 larvae (23%). Two larvae had intermediate patterns.

A. pseudoharengus larvae exhibit five ventral pigmentation patterns anterior to the pelvic region. One pattern (Fib. 1b) is that described for the species by Leim (1924) and occurred on 9 larvae (12% of 1977 specimens). A second pattern (Fig. 1c) is again similar to *A. aestivalis* and was present on 48 larvae (65% of 1977 specimens). A third pattern (Fig. 1d), however, is very similar to that reported by Leim (1924) for *A. sapidissima*. This pattern, consisting of a double curve in the rows of melanophores with the rows then drawing closer together at the posterior end, was observed on 9 larvae (12% of 1977 specimens). Ten larvae had intermediate patterns.

Two additional patterns were observed in 1981 collections of *A. pseudoharengus*. Sixteen larvae (80% of 1981 specimens) had a pattern beginning with 0-2 melanophores along the mid-line of the isthmus between the gills, followed by 2-4 diverging melanophores, a short break, then parallel rows of melanophores along the junction of the body and intestine. In the other pattern which occurred on 3 larvae (15% of 1981 specimens) the double row of melanophores converge, there is a short break, then the rows diverge. One larva observed had an intermediate pattern.

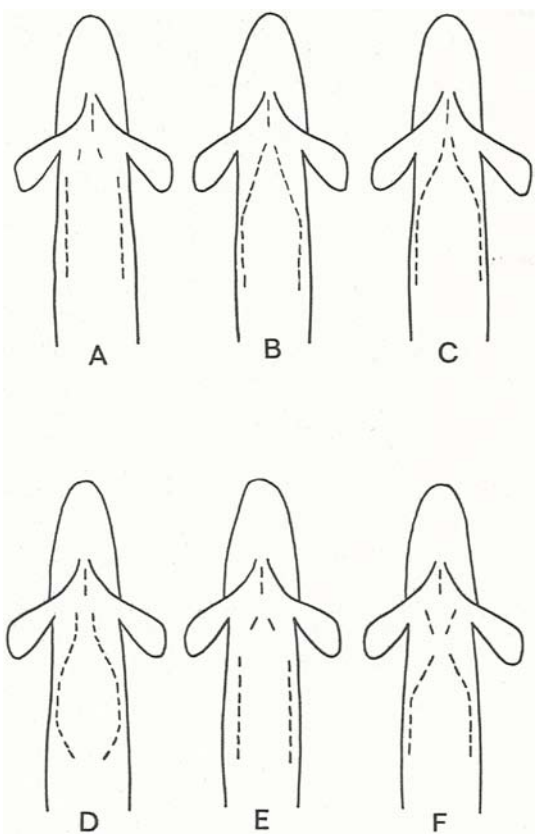

Fig. 1. (a) *A. aestivalis*, 6.7-12.7 mm SL;
(b) *A. aestivalis*, 10.2-10.8 mm SL;
A. pseudoharengus, 8.2-13.8 mm SL;
(c) *A. aestivalis*, 10.2-13.6 mm SL;
A. pseudoharengus, 10.8-18.1 mm SL;
(d) *A. pseudoharengus*, 16.5-19.1 mm SL;
(e) *A. pseudoharengus*, 7.1-12.0 mm SL;
(f) *A. pseudoharengus*, 10.0-12.1 mm SL.

DISCUSSION

The v-shaped pigment pattern described by Leim (1924) for alewives (he did not distinguish between A. aestivalis and A. pseudoharengus) is only one of many patterns observed in Potomac River populations of the two species. The more typical pattern for A. aestivalis is a line of melanophores along the isthmus, parallel double rows posterior to the cleithrum, a break, and then widely spaced parallel rows of melanophores.

One A. pseudoharengus pattern is very similar to that described for A. sapidissima. Other A. pseudoharengus patterns are nearly identical to those Mansueti (1962) described for putative A. mediocris larvae. In addition, collections made at the same location and same time of year, but in different years, yielded A. pseudoharengus with different pigmentation patterns. Figure 1c shows the more typical pattern among the 1977 specimens and Fig. 1e shows the typical 1981 pattern.

We conclude that ventral pigmentation patterns for larval A. aestivalis and A. pseudoharengus show such a degree of overlap and variation that this character is not reliable or even useful in separating Alosa species as has previously been thought. Time consuming methods based on meristics appear to be the only reliable means of separation.

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VARIATIONS IN VENTRAL MIDLINE MELANOPHORE COUNTS ON SOME CULTURED PACIFIC SCULPIN LARVAE

Jeffrey B. Marliave and Victor J. Elderton

ABSTRACT

Variation of melanophore counts was clarified through study of reared developmental series from known parental sources. Postanal ventral midline melanophores were counted in Ascelichthys rhodorus, Artedius fenestralis, Artedius lateralis, Clinocottus acuticeps, Enophrys bison and Oligocottus maculosus. Significant variation was found among species, between parental groups within a species, and between different age groups of siblings. Diminution in melanophore numbers in late larval stages was related to a particular phenomenon of apparent migration of melanophores. Erroneous identification of these and other larval species may arise from use of either small sample sizes or single parental groups for determining ranges of melanophore counts.

INTRODUCTION

Although ichthyoplankton studies have received increased emphasis in fisheries science, identification of certain species of marine fish larvae remains a problem. Laboratory rearing of larvae from known parental sources has been recommended as a solution to identification problems (Ahlstrom and Moser in press). Richardson (in press) reported that larvae of the Artedius-Clinocottus-Oligocottus group of cottids were among the most difficult to distinguish. Among the characters used for identification of these and other cottids is the count of postanal ventral midline melanophores (Richardson and Washington 1980). We have examined the variation for this character among species, parental groups within species, and age groups among siblings for six cottid species.

METHODS

Specimens of six cottid species were reared from known, unmixed parental sources at the Vancouver Public Aquarium (Table 1). Most of the laboratory culture was performed prior to the design of this study, so that more preserved material was availagle for some species than for others. Egg masses were collected from the field in advanced stages of embryonic development and were presumed to have experienced physical incubation conditions normal for their species. In all cases, larvae were hatched in 1,000 liter tanks, flat black in color, with overhead incandescent lighting yielding a maximum 600 lumens at the water surface, with through-flowing seawater (24-29 ppt salinity, 9-12°C) and were fed to excess daily with newly hatched Artemia salina nauplii. Since all larvae were reared under identical conditions of lighting and background color, any effects of rearing conditions on development of melanophores were expected to be uniform among different species and hatches. Fish larvae were preserved at intervals in 3% buffered formalin of 15 ppt salinity. Age was recorded as days post-hatching. For certain preservation ages from particular hatches, there were no comparable preservation ages from other hatches within the same species, so that these specimens could not be used for statistical analyses. Thus, the total ranges for melanophore counts in three species exceeded the ranges used in statistical analyses for those species (Table 1). Statistical comparisons were made with one-tailed analysis of variance, using the F test at the .05 level of significance.

RESULTS AND DISCUSSION

Significant variation exists in ventral midline melanophore counts for 0-day larvae among all the different species, including the two Artedius species (Figure 1). These differences in melanophore counts for 0-day larvae could not be artifacts of any laboratory rearing conditions, as all these specimens were killed from new hatches from egg masses which had incubated under natural conditions in the field. It should be noted from Table 1 (for larvae of all ages), however, that for all species the total ranges of melanophore counts overlap to a greater or lesser degree, thus limiting the diagnostic value of such counts.

Within each species, significant variation occurred among counts on 0-day larvae from different parental groups (egg masses), with the exception of the two small-sized samples of 0-day Clinocottus acuticeps (Figure 1). Again, it must be noted that ranges for selected hatches may be similar, whereas other hatches may markedly differ in both ranges and means. This finding indicates that caution must be exercised in utilizing melanophore count data based on reared larvae from single parental sources. Overall, only E. bison and O. maculosus regularly yielded counts different from the other species (E. bison counts lower and O. maculosus higher). For O. maculosus, however, it should be noted (Table 1) that lower ranges of counts are reported for more southerly populations (Stein 1973, B.B. Washington personal communication).

There also was significant variation among those counts on older larvae of A. rhodorus from separate

Table 1. Cottid species used for analysis of variance in ventral midline melanophore counts, and ranges of such counts.

Species	No. Egg Masses	Total No. Larvae	Total Range, All Specimens	Range, Specimens Used for ANOVA	Ranges Reported in Other Studies
Ascelichthys rhodorus	10	817	6-37	6-32	11-28[1]
Artedius fenestralis	2	108	6-32	6-32	13-24[2]
Artedius lateralis	3	273	7-39	7-39	13-24[2]
Clinocottus acuticeps	N/A	167	6-32	10-29	4-9[2]
Enophrys bison	2	106	2-18	2-18	10-14[2], 3-16[3]
Oligocottus maculosus	6	194	17-49	19-47	11-20[4], 16-20[5]

1 - Matarese and Marliave 1981, 2 - Richardson and Washington 1980, 3 - Misitano 1978, 4 - Stein 1973,
5 - B.B. Washington personal communication

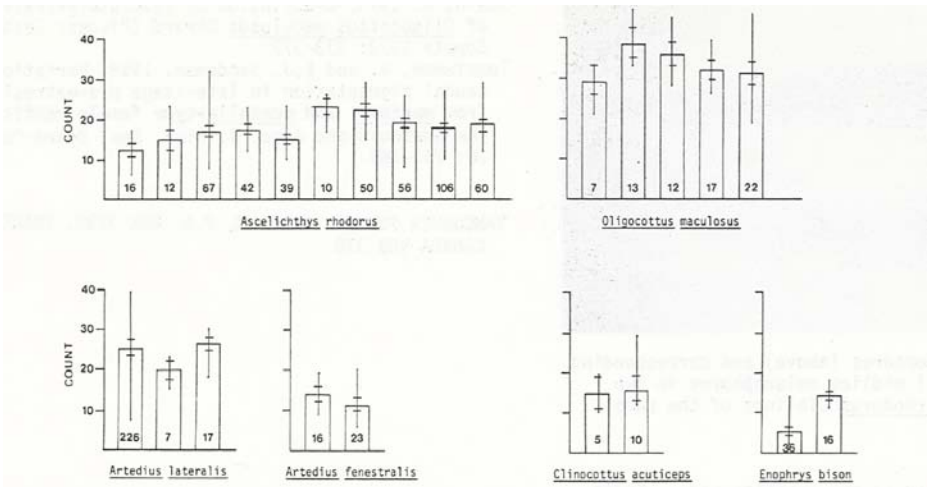

Figure 1. Postanal ventral midline melanophore counts on larvae of 0-days age from different parental sources; histogram bars indicate mean values; vertical lines, ranges; pairs of horizontal dashes, 95% confidence limits; and numbers at bases of histogram bars, sample sizes. Each bar represents a different parental source.

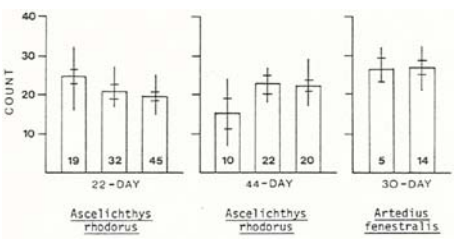

Figure 2. Postanal ventral midline melanophore counts on postflexion larvae from different parental sources: means, ranges, 95% confidence limits and sample sizes indicated as in Figure 1.

hatches (Figure 2), but not between 30-day larvae from two hatches of A. fenestralis. Unfortunately, older larvae of similar age were not available for analysis from the other species.

Within sibling groups (hatches), there were statistically significant developmental changes in melanophore counts (Figure 3) for three species (A. rhodorus - 3 hatches, E. bison - 1 hatch, A. fenestralis - 2 hatches), but no significant changes were detected for O. maculosus (2 hatches). With the O. maculosus, however, samples were only available for preflexion larvae, so changes might occur over longer developmental periods in this species. Generally speaking, newly hatched larvae tend to have low counts which increase during early development, then decrease as the larvae approach metamorphosis (Figure 3).

No explanation exists for the early developmental increase in counts, other than that the melanin may not be fully elaborated in some pigment cells. The decreases in counts during later development, a possibility suggested by Richardson and Washington (1980) were observed to have resulted from an apparent vertical migration along myosepta of melanophores, which later disappeared, leaving gaps in the ventral midline row of melanophores (Figure 4). Elevated melanophores were observed in certain individuals and gaps in corresponding positions observed in siblings of the same and older ages. This phenomenon of melanophores elevated above the ventral midline in areas where gaps later appeared was observed in all species except E. bison and A. lateralis, although gaps were detected in some E. bison.

Another phenomenon, observed in A. rhodorus, occurred as the posterior trunk musculature grew broader. Ventral midline melanophores generally split into a double row, but some only migrated to one side or the other, and

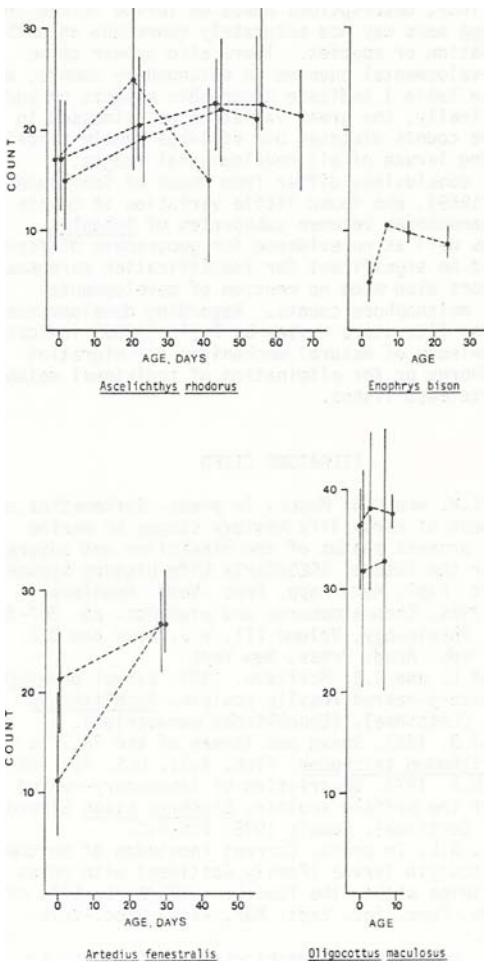

Figure 3. Developmental changes in ventral midline melanophore counts within parental sources; dots and vertical lines indicate means and ranges; samples from the same parental source are connected by dashed lines.

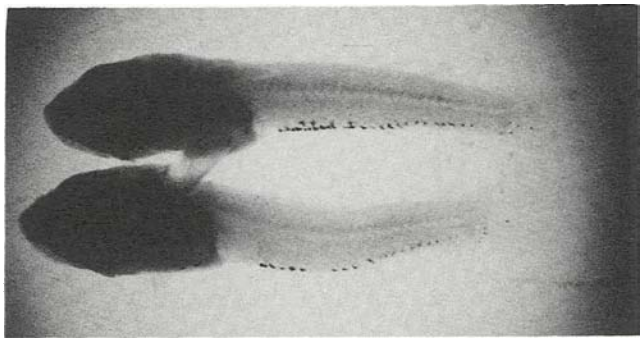

Figure 4. Elevated melanophores (above) and corresponding gaps (below) among ventral midline melanophores in two postflexion Ascelichthys rhodorus siblings of the same size, reared together.

corresponding gaps appeared on the opposite side. Splitting of ventral midline melanophores into a double row during late larval development has been observed in Trichodon trichodon (Marliave 1981).

CONCLUSIONS

The results of this study indicate that ventral midline melanophore counts vary considerably among parental sources. Thus, descriptions based on larvae reared from a single egg mass may not accurately represent an entire fish population or species. There also appear to be regular developmental changes in melanophore counts, and the data in Table 1 indicate geographic effects on such counts. Finally, the great variation we witnessed in melanophore counts dictates use of large sample sizes representing larvae of all developmental stages.

These conclusions differ from those of Templeman and Sandeman (1959), who found little variation in counts of caudal melanophores between subspecies of Sebastes marinus, as well as no evidence for geographic differences which could be significant for identification purposes. These authors also made no mention of developmental changes in melanophore counts. Regarding developmental changes, the literature review by Fujii (1969) indicates little knowledge of natural mechanisms for migration of melanophores or for elimination of individual melanophores in teleost fishes.

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EFFECTS OF TEMPERATURE ON EARLY DEVELOPMENT OF RED DRUM (SCIAENOPS OCELLATA)

JOAN HOLT & CONNIE ARNOLD

ABSTRACT

Successful development of eggs and larvae of laboratory spawned red drum was limited to temperature above 20 C. Red drum are fall spawners and temperatures of 20 C or less may be encountered during some years. Year to year variation in year classes are commonly observed and may be partly explained by narrow temperature tolerance of the larvae. Studies of development and growth were undertaken to identify the age at which red drum larvae can tolerate low temperature, and to examine the effects of various temperatures on growth throughout the larval period.

Red drum were spawned at the UT PAML[1] laboratory by mainpulations of the temperature and photoperiod cycles to simulate natural seasonal changes. Larvae reared in 1000 ml experimental chambers were initally fed rotifers (Brachionus plicatilis). Artemia salina nauplii were fed to 8 day old larvae (Fig. 1).

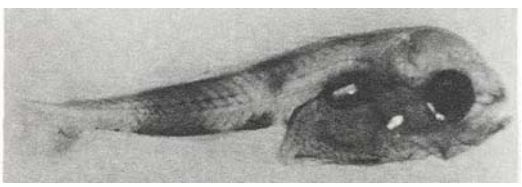

Fig. 1. Eight day old red drum larvae.

Red drum were hatched and maintained at test temperatures for two weeks in experiment 1 (Fig. 2). In experiment 2 larvae maintained at 25 C until day 11, were changed to the test temperatures for two weeks. Growth was measured as increase in standard length per day. Growth rates increased with increasing temperature (within the range tested) and with age.

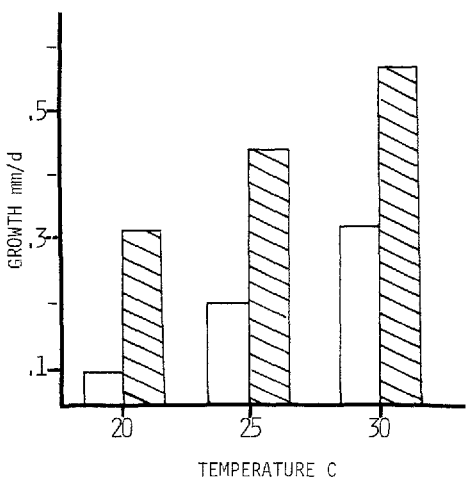

Fig. 2. Growth of red drum larvae at 3 test temperatures. Experiment 1 is shown by the clear bars and experiment 2 by the cross hatched bars.

Red drum larvae were hatched at 25 C and changed to 20 C on subsequent days; after two weeks, measurements of standard length were made. Control fish remained at 25 C for two weeks (Fig. 3).

There was a significant correlation ($p < 0.01$) between growth rate and age of first exposure to 20 C. Growth rates of larvae exposed to 20 C at day 9 were equivalent to controls at 25 C. Survival rates were greatly increased when larvae were maintained at 25 C through the yolk-sac stage before being exposed to 20 C.

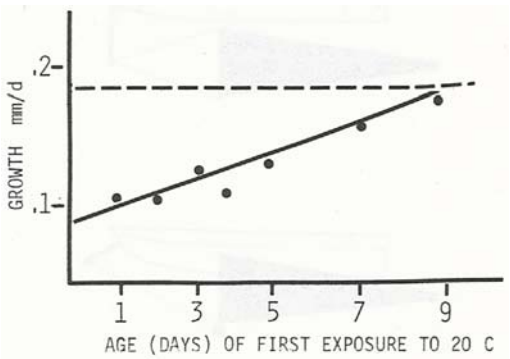

Fig. 3. Growth of red drum larvae as a function of age of first exposure to 20 C.

CONCLUSIONS

1. The low survival rate of the early stages of red drum in non-optimal temperatures improved with age.
2. Survival rates were greatly increased when larvae were maintained at 25 C through the yolk-sac stage and first feeding (3 days) before being exposed to 20 C.
3. Growth rate of all ages increased with increasing temperature; length of two-week-old larvae at 30 C was three times that of larvae at 20 C.
4. During the first month, larvae held at 20 C 70% of the time grew at rates equivalent to larvae continuously exposed to 20 C.

[1]UNIVERSITY OF TEXAS MARINE SCIENCE INSTITUTE, PORT ARANSAS MARINE LABORATORY, PORT ARANSAS, TEXAS 78373.

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COMPARATIVE EFFICIENCIES OF 505 AND 800 MICRON MESH NETS FOR LAKE ICHTHYOPLANKTON

Robert D. Hoyt and Dennis L. Webb

ABSTRACT

Larval fishes were sampled in Rough River Lake, Kentucky, in the spring and summer of 1980 using ichthyoplankton nets of 505 and 800μ mesh sizes. Nets of different meshes were mounted on each side of the bow of the boat (Fig. 1), and sampled surface strata only. Samples were made weekly from March 28 to July 24, 1980, and twice weekly from April 18 to May 30. Of 152,454 larvae collected, significantly more were taken with 505 mesh than with the 800 (Fig. 2). Of 16 species observed, the bluntnose minnow, channel catfish, banded sculpin, freshwater drum, and an unidentified darter were taken only in the 505 μ and not the 800 μ gear. Greater numbers of larvae per 100 m^3 of water sampled were taken with the smaller mesh throughout most of the study and with the larger mesh in the latter weeks (Fig. 3). Average total lengths of representative individuals of the six most abundant taxa collected were greater for specimens collected in the larger mesh on each sampling date (Fig. 3). This study was supported by the National Marine Fisheries Service, NOAA, and the Kentucky Department of Fish and Wildlife Resources, under PL 88-309, Project Number 2-358-R.

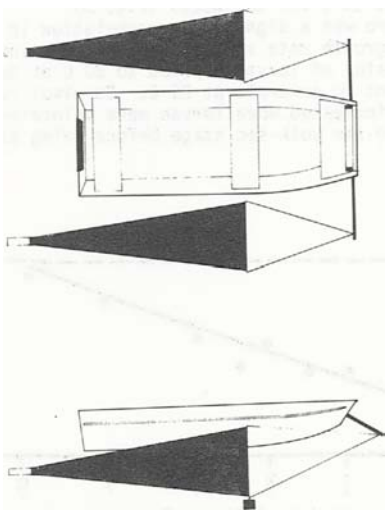

Fig. 1. Dorsal and lateral diagrams of net mountings.

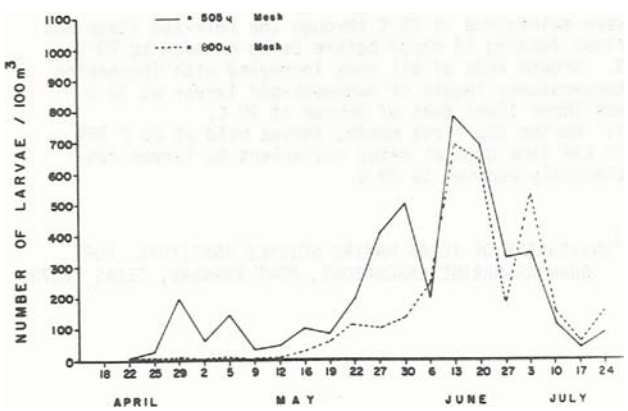

Fig. 2. Total catch using 505 μ and 800 μ mesh nets.

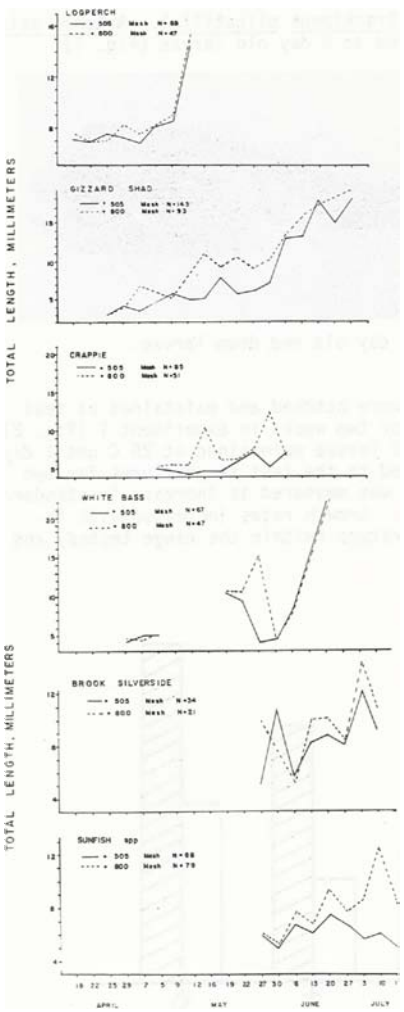

Fig. 3. Average TL of 6 most abundant taxa.

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SEASONAL SPECIES COMPOSITION AND BIOMASS ESTIMATES OF LARVAL AND JUVENILE FISHES FROM NORTH INLET, SOUTH CAROLINA

Lynn Barker and Richard H. Moore

ABSTRACT

A year-long series of ichthyoplankton collections was undertaken in North Inlet, South Carolina to determine seasonal patterns in species occurrence, relative abundance, and diversity among larval, post-larval and juvenile fishes in a tidal inlet. Over 200 five-minute samples were taken over a 2-week period at three sample transects during each season in 1979. Collections were made using a 0.5-meter 505 μ mesh conical net suspended from a vertical guy-wire. Depth integrated samples were obtained by lowering and raising the net during the 5-minute sample period. Sample volumes were determined by means of a flowmeter in the mouth of each net. Figure 1 illustrates that ichthyoplankton is dominated in the fall and winter by sciaenids and secondarily sparids. During the warmer months engraulids make the greatest contribution to numbers and biomass, although in the summer gobiids are also important. It is possible that prevailing northeasterly winds and storm conditions were responsible for the similarity between North and South Jones Creeks in the winter. In the fall, South Jones differed from North Jones and Town Creeks by continuing to display a "summer" ichthyoplankton complement well after the other creeks had changed to their "winter" sciaenid-dominated assemblages. These data illustrate a seasonal transition in the ichthyofauna for the creeks sampled. Inter-creek variations are attributed to differences in geographic location within the estuary, water depth, substrate type, and water velocity.

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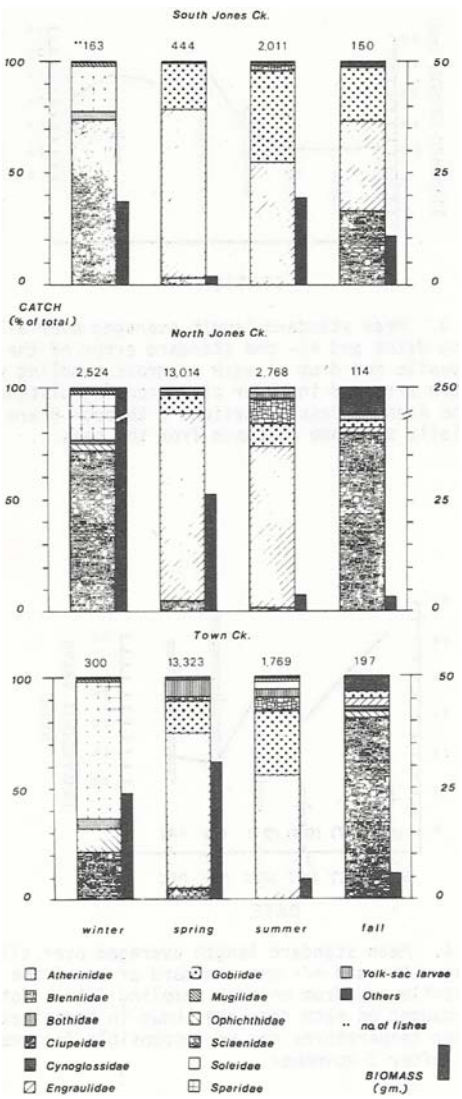

Fig. 1. Bar graph illustrating seasonality, catch, and biomass for larval and juvenile fishes collected in three tidal creeks.

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DISTRIBUTION AND ABUNDANCE OF EGGS, LARVAE AND JUVENILES OF RED DRUM (Sciaenops ocellatus) IN SEAGRASS BEDS IN A SOUTH TEXAS ESTUARY

Scott Holt and Connie Arnold

ABSTRACT

Red drum eggs, larvae, and juveniles were collected in or near seagrass beds in Redfish Bay, Texas from September through December, 1980. Red drum were collected in Halodule and Thalassia beds with a benthic sled and a 1.0 mm mesh zooplankton net. Red drum eggs and newly hatched larvae were observed moving into the grassbeds with tidal currents. Abundance of larval and juvenile red drum in the grass beds ranged from none up to 1.0 per M^2. Red drum were captured in both day and night samples but more fish were consistently taken in day samples than in night samples. The smallest fish were taken at sites nearest the Aransas Pass. Differences in abundance between sample sites could be related to density of seagrass blades and water depth. Differences in abundance over time could be related to temperature and seasonal changes in water depth. Juvenile red drum moved out of the seagrass beds in late November with the onset of cold (< 16°C) water temperatures.

Figure 1. Location of sampling sites in Redfish Bay.

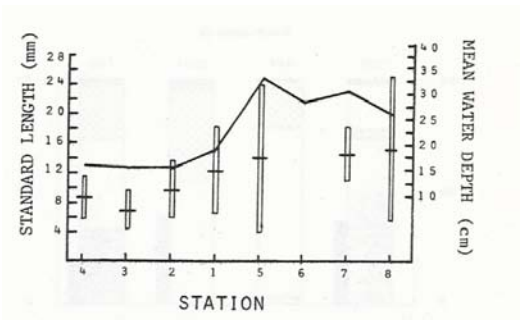

Figure 3. Mean standard length averaged over all sampling dates and +/- one standard error of the mean for juvenile red drum at each seagrass sampling site. Sites are arranged in order of increasing distance from the Aransas Pass. Stations 5 through 8 are all essentially the same distance from the pass.

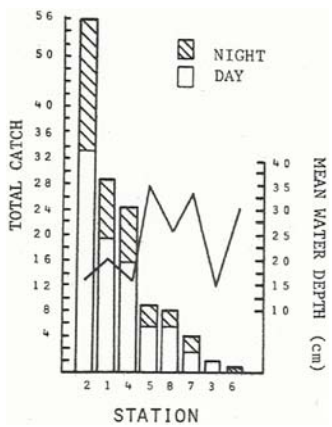

Figure 2. Total catch of juvenile red drum at each seagrass sampling site during the period 23 September to 2 December 1980. Mean water depth is generally inversely related to total catch. The low catch at site 3 is due to relatively higher current speeds at that site compared to other sites.

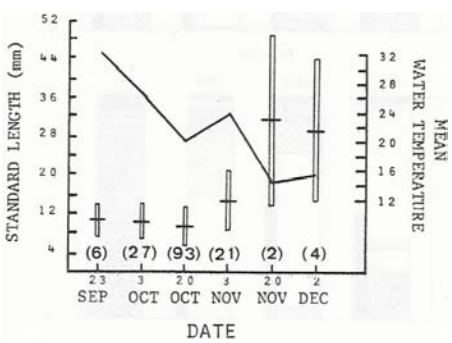

Figure 4. Mean standard length averaged over all sampling sites and +/- one standard error of the mean for juvenile red drum on each sampling date. Total number caught on each date are shown in parenthesis. Low water temperatures may be responsible for small catches after 3 November.

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